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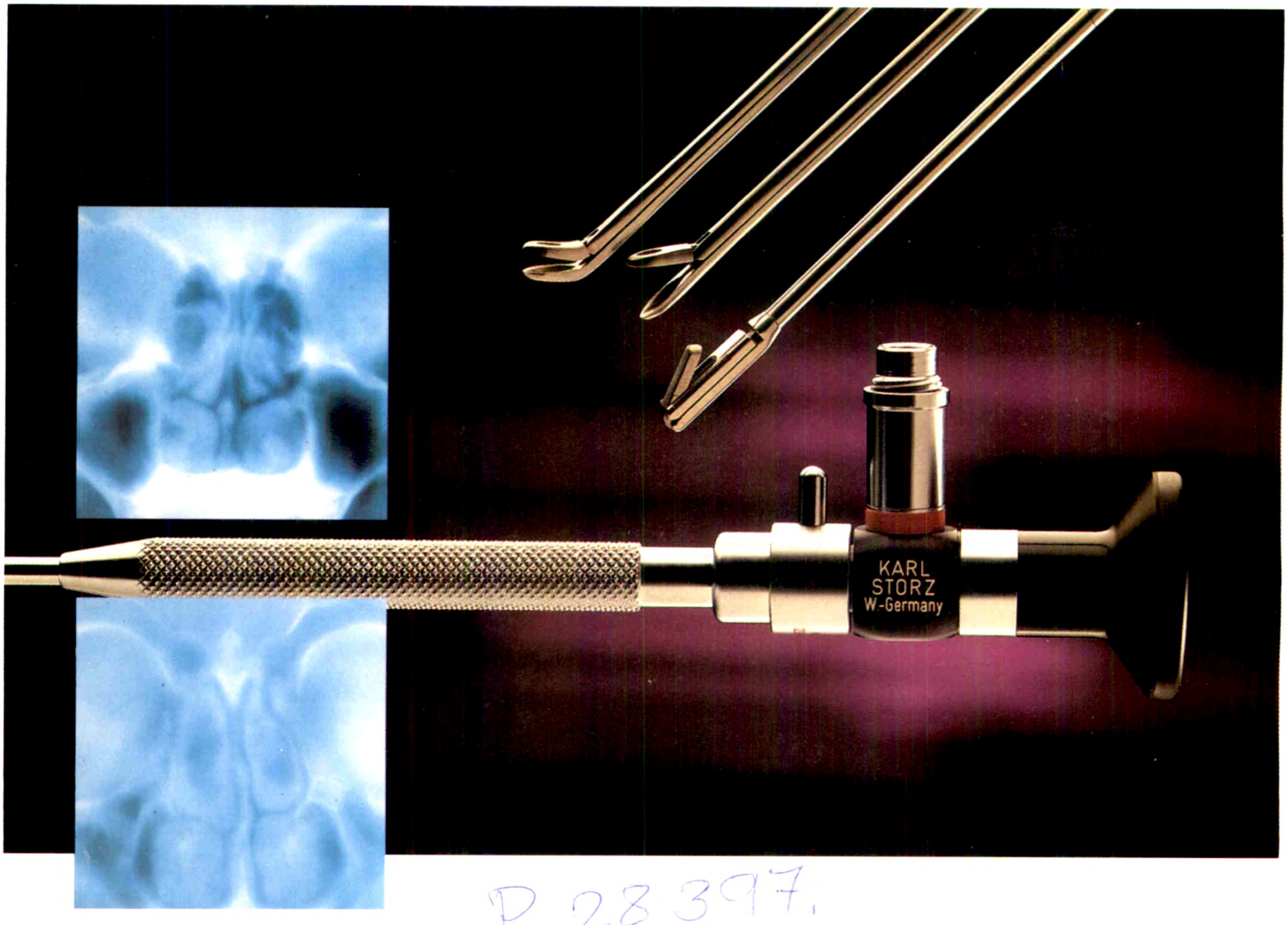

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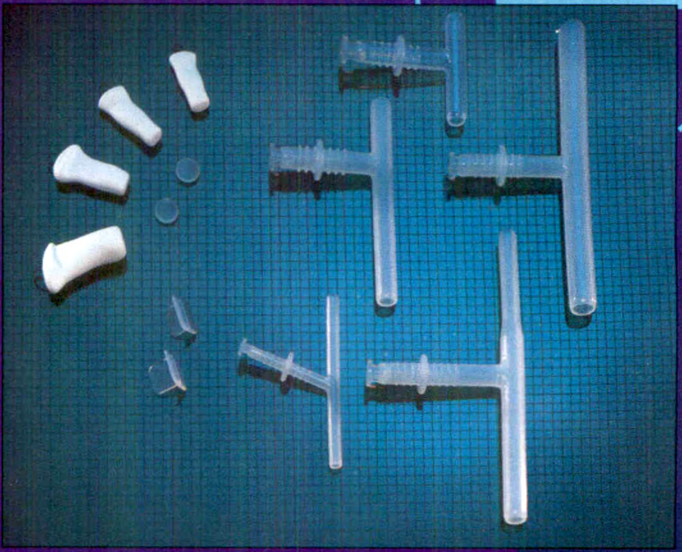

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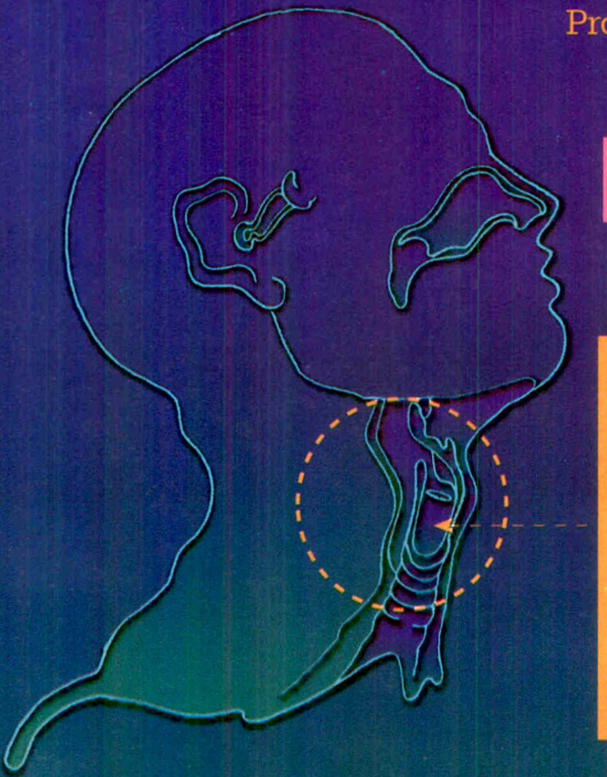

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EFFECTS OF OTITIS MEDIA ON EXTENDED HIGH-FREQUENCY HEARING IN CHILDREN

ROBERT H. MARGOLIS, PHD
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Extended high-frequency (EHF) hearing was studied in children with and without histories of chronic or recurrent otitis media (OM). The EHF thresholds were found to have good test-retest repeatability. Children with OM histories had poorer EHF hearing than children without OM histories. The EHF hearing in OM children appeared to be related to OM severity. Children with residual tympanometric abnormalities had poorer EHF hearing than OM children with normal middle ear function. The results suggest evidence for middle ear and inner ear components of EHF hearing losses in children with OM.

KEY WORDS — hearing, otitis media.

A number of studies have suggested that sensorineural hearing loss may be a sequela of otitis media (OM). Clinical studies have demonstrated elevated bone conduction thresholds in patients with chronic or recurrent OM.[1-6] A few studies have demonstrated other auditory findings that suggest the presence of inner ear disease.[7,8] Laboratory studies have shown a variety of histopathologic and electrophysiologic abnormalities in animals that suggest inner ear damage secondary to OM.[9,10] Diffusion of toxins across the round window membrane has been demonstrated in many laboratory studies and inferred from human temporal bone histology.[11,12]

Because frequency coding along the basilar membrane progresses from high to low frequencies with distance from the basal end of the cochlea, sensorineural hearing loss produced by diffusion of OM-related toxins across the round window membrane would be expected to preferentially affect high-frequency hearing. Previous studies have demonstrated a relationship between OM and hearing loss in the extended high-frequency (EHF) range (8 to 20 kHz).[13,14] The possibility exists, however, that residual middle ear disease may also produce EHF hearing loss. Previous studies have not evaluated middle ear function to rule out this possibility.

This investigation was undertaken to

1. Obtain normative data for EHF hearing thresholds in 3- to 10-year-old children.
2. Evaluate test-retest reliability of EHF hearing thresholds in normal children.
3. Study the effects of OM on EHF hearing in children with various residual middle ear conditions, evaluated by multifrequency tympanometry.

In this preliminary report, we present results from a normal group of 3- to 7-year-old children and a group of children being followed for chronic or recurrent OM.

METHODS

Subjects. Normal children were recruited from two pediatric clinics by using the following selection criteria: 1) continuous medical care provided at the clinic since birth, 2) two or fewer documented episodes of OM since birth, 3) no documented OM in the past year, and 4) normal hearing in the conventional frequency range (≤15 dB hearing level at 250 to 8,000 Hz). The study design calls for 10 children in each 1-year age interval from 3 to 10 years. In this preliminary report, data are presented for 41 ears of 21 subjects 3 to 7 years old. Mean conventional audiograms for these subjects, divided into three age groups, are shown in Fig 1. There is a small, statistically significant ($p = .004$) improvement in hearing as a function of age. This age effect may reflect hearing sensitivity changes, or it may result from differences in response criterion.

The OM group consisted of a subset of subjects being followed for chronic or recurrent OM. These children, described in more detail elsewhere,[15] are seen every 3 months for a minimum of 3 years after myringotomy with tympanostomy tube placement. All measurements reported in this study were made after tube extrusion and after complete healing of the tympanic membrane. To minimize hearing losses due to middle ear disease, subjects were selected with

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From the Department of Otolaryngology, Otitis Media Research Center, University of Minnesota, Minneapolis, Minnesota. Supported by grant P01-DC00133 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

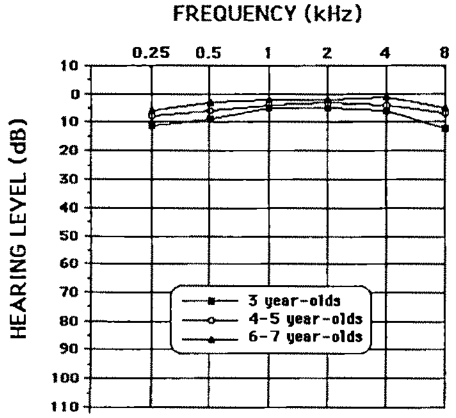

Fig 1. Conventional-frequency audiometric thresholds for three groups of children who have had no more than two documented episodes of otitis media since birth.

no otoscopic evidence of current OM, normal hearing in the conventional frequencies (≤15 dB hearing level 250 to 8,000 Hz), and no air-bone gap (≤5 dB at all frequencies 250 to 4,000 Hz). Data are reported on 49 ears of 28 children 3 to 7 years of age.

Audiometry and Tympanometry. Each subject was tested by conventional air and bone conduction audiometry, multifrequency tympanometry, and EHF audiometry.

Conventional audiometry was performed with a clinical audiometer (Virtual model 320) equipped with standard air conduction and bone conduction transducers (Telephonics TDH-50P and Radioear B-71, respectively). Air conduction and bone conduction sections of the audiometer were calibrated by standard methods (ANSI S3.6-1989 and ANSI S3.26-1981, respectively[16,17]). Extended high-frequency audiometry was performed with the same audiometer with headphones designed for high-frequency efficiency. The headphones (Koss Pro/4X Plus) were modified by the audiometer manufacturer by replacement of the circumaural cushion and disconnection of the low-frequency transducer in the earphone. The EHF section of the audiometer was calibrated by a sound level meter with the earphone placed on a 6-cm^3 flat plate coupler. Since standard reference levels do not exist for EHF audiometry, results are expressed in sound pressure level (decibels re: 20 µPa). The EHF thresholds were obtained by the standard method for manual pure tone audiometry (ANSI S3.21-1978[18]).

Multifrequency tympanometry was performed with a computer-controlled acoustic immittance system (Virtual model 310) that provides probe frequencies ranging from 226 to 2,000 Hz. Tympanograms were recorded over the entire range of probe frequencies in 1/6-octave intervals. The instrument produces a fixed ear canal air pressure, sweeps the probe tone, and obtains complex acoustic admittance measurements at each probe frequency. This process is repeated for ear canal pressures ranging from 400 to −500 decapascals. The instrument is calibrated in calibration cavities (0.5, 2.0, and 5.0 cm^3) as specified by the American National Standard related to acoustic immittance instruments (ANSI S3.39-1987[19]).

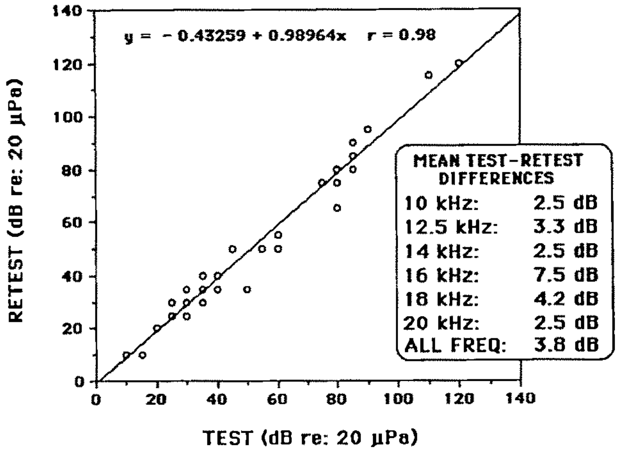

Fig 2. Test-retest scatterplot of extended high-frequency thresholds from six ears of three children. Values in inset are mean absolute values of test-retest differences.

RESULTS

Test-retest Reliability. Test-retest EHF thresholds for six ears of three children (ages 61, 63, and 67 months) are shown in Fig 2. The mean test-retest difference for all test frequencies combined was 3.8 dB, and the reliability coefficient was .98. Test-retest differences in the EHF range are only slightly larger than those in the conventional audiometric frequencies.[20] Although ear canal sound pressure measurements show considerable variability in this frequency region,[21] this variability does not appear to result in poor test-retest reliability. Hearing sensitivity is probably the result of a vector summation of energy over the effective portion of the tympanic membrane, which is not as variable as probe tube sound pressure levels.

Normal Extended High-frequency Thresholds. Figure 3 presents mean EHF thresholds for the normal subject group, separated into three age groups. Similar to the conventional frequency range, there was a trend for thresholds to improve with age. The effect did not reach statistical significance at the .05 confidence level (p = .09).

Figure 4 presents mean EHF thresholds for the normal group and data from Fausti et al,[20] who obtained thresholds from a group of young adults (ages 18 to 25 years) using similar instrumentation. In general, the data are in very close agreement. There appeared to be a frequency region (11 to 14 kHz) in

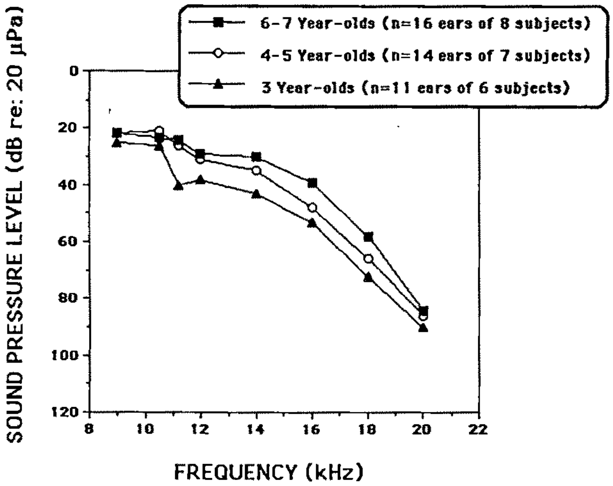

Fig 3. Mean extended high-frequency thresholds for 3- to 7-year-old children divided into three age groups. There was systematic effect of age, with auditory thresholds decreasing with age.

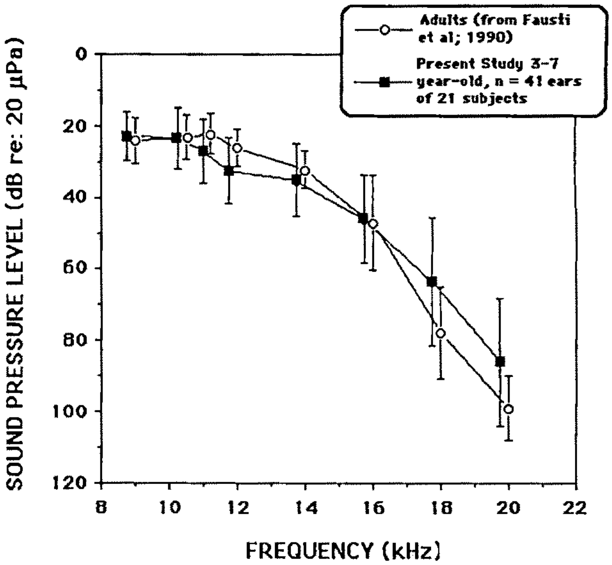

Fig 4. Mean extended high-frequency thresholds (±1 SD) for 3- to 7-year-old children and for young adults (ages 18 to 25 years). Adult data are from Fausti et al.[20]

which the older group had slightly better hearing, and another (>16 kHz) in which the younger group had better hearing.

Extended High-frequency Hearing in Children With Otitis Media. Figure 5 presents EHF hearing thresholds for the normal children and for children with histories of chronic or recurrent OM. The OM group was separated by tympanometric results in order to explore the possible effects of residual middle ear disease. Tympanometric results were classified as normal or abnormal based on 226-Hz admittance magnitude tympanograms and multifrequency tympanograms. Criteria for grouping subjects according to tympanograms are presented in the Table.[22,23] An analysis of variance indicated that the differences among groups were significant (p = .0001). Post-hoc analyses revealed that the normal group was significantly different from all other groups (p = .002, .03, and .0001 for groups 2, 3, and 4, respectively). No other comparisons reached statistical significance at the .05 level. A comparison of groups 2 and 3 pooled against group 4 yielded a significance level of .09, suggesting a trend for those with abnormal 226-Hz

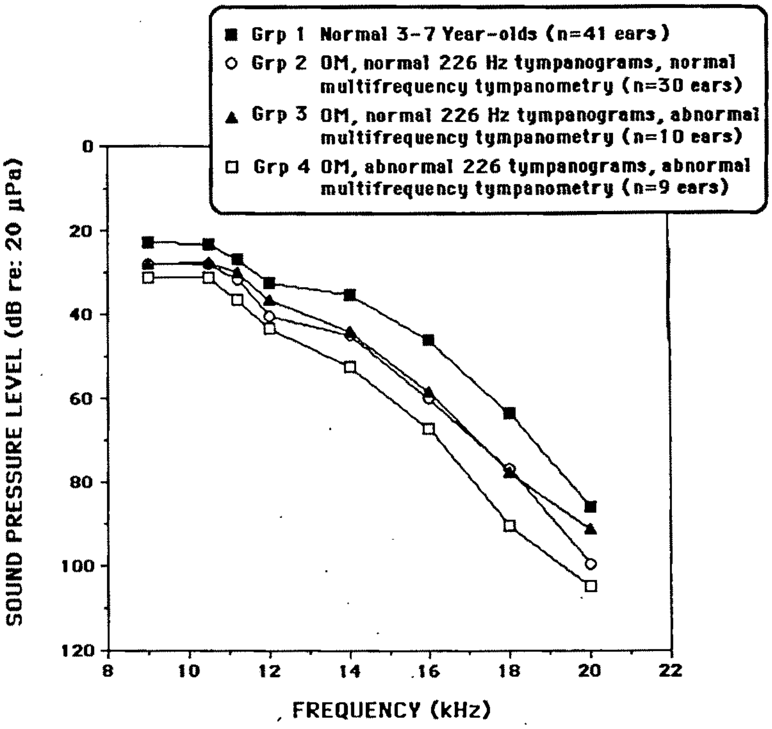

Fig 5. Mean extended high-frequency thresholds for normal 3- to 7-year-old group (group 1) and for three groups of children with histories of chronic or recurrent otitis media (OM). Otitis media subjects are divided into three groups based on tympanometry as follows: normal 226-Hz and multifrequency tympanograms (group 2); normal 226-Hz tympanograms and abnormal multifrequency tympanograms (group 3); abnormal 226-Hz and multifrequency tympanograms (group 4). Otitis media children had normal hearing in conventional-frequency range, no air-bone gaps, and no otoscopic evidence of active OM at time extended high-frequency threshold measurements were made.

CRITERIA FOR OTITIS MEDIA GROUPS BY TYMPANOMETRY RESULTS

	*226-Hz Tympanogram**	*Multifrequency Tympanograms*†
Normal control group		
Group 1	Normal	Normal
History of otitis media		
Group 2	Normal	Normal
Group 3	Normal	Abnormal
Group 4	Abnormal	Abnormal

All groups with history of otitis media had no otoscopic evidence of it at time of testing, had normal hearing in range 250 to 8,000 Hz (≤15 dB hearing level), and had no air-bone gap exceeding 5 dB in range 250 to 4,000 Hz.

*Normal if *static admittance* (compensated by using negative tail) was 0.22 to 0.81 millimhos and if tympanometric width (gradient), compensated at +200 decapascals, was 59 to 151 decapascals. Ranges are from previously published normative study.[22]

†Normal if shape followed model of Vanhuyse et al[23] and if resonance (susceptance notch ≤ positive tail) occurred in range 800 to 1,600 Hz.

tympanograms to have poorer hearing than subjects with normal 226-Hz tympanograms.

In order to explore the effect of OM severity on high-frequency hearing, OM subjects with normal 226-Hz tympanograms were classified as having mild or severe OM on the basis of frequency of OM at the time of their quarterly study visits. Children who had OM at 25% or fewer of study visits were classified as having mild OM. Those with OM at more than 25% of study visits were classified as having severe OM. Figure 6 suggests that there is a trend for children with more severe OM to have poorer high-frequency hearing. The difference did not reach statistical significance at the .05 level ($p = .07$).

DISCUSSION

Our experience with EHF audiometry indicates that the technique provides a reliable, accurate method for evaluating high-frequency hearing. Past concerns regarding reliability and stability appear to be manageable, provided that proper calibration methods are employed.

Children with histories of OM have poorer high-frequency hearing than children without frequent middle ear disease. The severity of OM may be related to high-frequency hearing. Because of the intersubject variability, more subjects will be needed to demonstrate this relationship to a statistical certainty.

The relative contributions of middle ear and inner ear changes to these high-frequency hearing deficits are difficult to parcel out. Several steps were taken in this investigation to control for residual middle ear disease. First, subjects were eliminated if they had otoscopic evidence of active OM. Second, OM subjects were selected who had no air-bone gaps in the conventional frequency region. Third, OM subjects were separated by tympanometry in an attempt to evaluate the likelihood of residual middle ear disease. Subjects with normal 226-Hz tympanograms and normal multifrequency tympanometry have the least likelihood of residual mechanical disturbances of the middle ear. These subjects had poorer hearing than their normal counterparts at all frequencies tested. Those with normal 226-Hz tympanograms and abnormal multifrequency tympanometry did not differ from those with normal multifrequency results. These abnormalities in the multifrequency tympanograms were predominantly characterized by high admittance and low resonant frequency, probably because of monomeric tympanic membranes. Monomeric tympanic membranes are not likely to affect high-frequency hearing, because the region of the eardrum that is likely to be scarred contributes little to ossicular chain vibration at high frequencies.[24] The group with abnormal 226-Hz tympanograms tended to have the poorest high-frequency hearing, perhaps reflecting a residual middle ear component as well as an inner ear component.

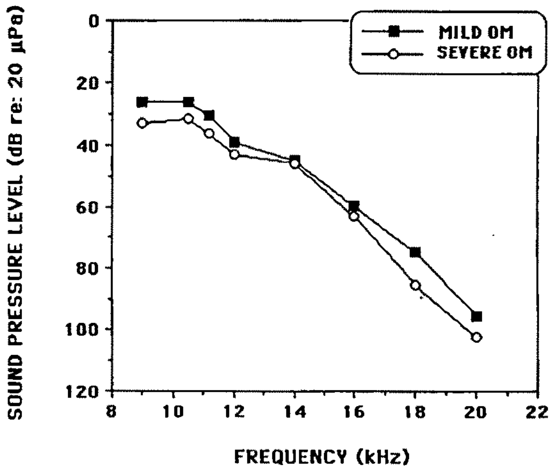

Fig 6. Mean extended high-frequency thresholds for otitis media (OM) subjects classified according to severity.

SUMMARY AND CONCLUSIONS

Extended high-frequency audiometry is a reliable method for evaluating high-frequency hearing in children. Children with OM histories had poorer EHF hearing than children without OM histories. There may be both an inner ear component and a middle ear component of the high-frequency hearing loss. Further studies employing larger subject groups are under way to further study high-frequency hearing in OM. The use of high-frequency bone conduction threshold measurements may be helpful for evaluating the role of residual middle ear disease in high-frequency hearing.

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THE JERUSALEM SYMPOSIUM ON SURGERY OF THE SKULL BASE AND ADJACENT MIDLINE REGION

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OLFACTORY EVOKED POTENTIALS AND CONTINGENT NEGATIVE VARIATION SIMULTANEOUSLY RECORDED FOR DIAGNOSIS OF SMELL DISORDERS

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Objective diagnosis of olfaction can be performed by registration of cortical olfactory evoked potentials (OEP) and of contingent negative variation (CNV). The CNV is a negative voltage developing at the vertex after discrimination of one of two smells while the patient is expecting a second stimulus. By an adequate procedure, including a long time window for averaging (2.56 seconds) with appropriate filters, the two tests can be performed simultaneously in a single session of less than 10 minutes. Anosmia is determinable by both OEP and CNV, although CNV shows less variability. On the other hand, CNV requires attention and some cooperation of the patient. Parosmia is accessible by CNV only; two odor qualities presented in random order have to be distinguished. Hyposmia can also be detected; just above the discrimination threshold, CNV amplitudes tend to be large — even enhanced — whereas OEP amplitudes may still be undetectable.

KEY WORDS — contingent negative variation, hyposmia, olfactory evoked potentials, parosmia.

INTRODUCTION

Basic attempts at the recording of olfactory evoked potentials (OEP) in humans were made by Finkenzeller[1] and by Allison and Goff.[2] The application of OEPs for clinical diagnosis was introduced by us[3] and by Herberhold.[4] The main influences of stimulus parameters were examined by several authors.[5-7]

The indication of hyposmia or anosmia can be obtained from OEP amplitude evaluation.[8,9] With uneasy patients, however, the extraction of clear potentials may be difficult, because the onset of an olfactory compared to an acoustic stimulus is much less precise, and the tolerable number of stimuli is smaller than that presented for cortical auditory evoked potentials. In addition, there is no way to judge parosmia by OEP, because the patient's sensation of a different odor is not expressed in the shape of the potential.

The contingent negative variation (CNV) does not require a steep stimulus onset and can rather reproducibly be recorded also from less calm patients, as long as they are attentive. The CNV is a negative voltage at the vertex developing after discerning one of two odor stimuli while expecting a second (auditory or visual) confirmative stimulus. By alternating presentation of two different odors, one of which is marked by the confirmative stimulus, the ability of odor discriminating can be examined.[10] A major problem in objective olfactometry has been the amount of time required for the necessary number of repetitions or parameter variations, because patients grow uneasy and adaptation may make interpretation of results questionable.

In this paper the time-saving simultaneous recording of OEP and CNV is demonstrated for patients with various smell disorders, in order to define and compare the applicability of the two methods for diagnosis of hyposmia, anosmia, and parosmia.

METHODS

Odor stimuli are presented in an electrically shielded, well-ventilated room. They are generated by our slightly modified impulse olfactometer.[8] During spontaneous inhalation one of five selectable odors is embedded without pressure change into the fresh airstream (Fig 1). As the fresh air immediately cleans the tubes and valves and the nose, olfactory adaptation is rendered very slight.[11] The sound produced by the magnetic valves is masked by broadband noise delivered via earphones at 65 dB hearing level (HL) in order to avoid an additional auditory evoked response. Through the same earphone the confirmatory stimulus is presented. Usually it is a 1-kHz tone (60 dB HL) that is to be terminated by pressing a button as soon as possible. This manual reaction has proved to increase the attention necessary for larger CNV amplitudes. Reaction time is observed as a measure of the patient's attention.

In this examination lilac and camphor were used at

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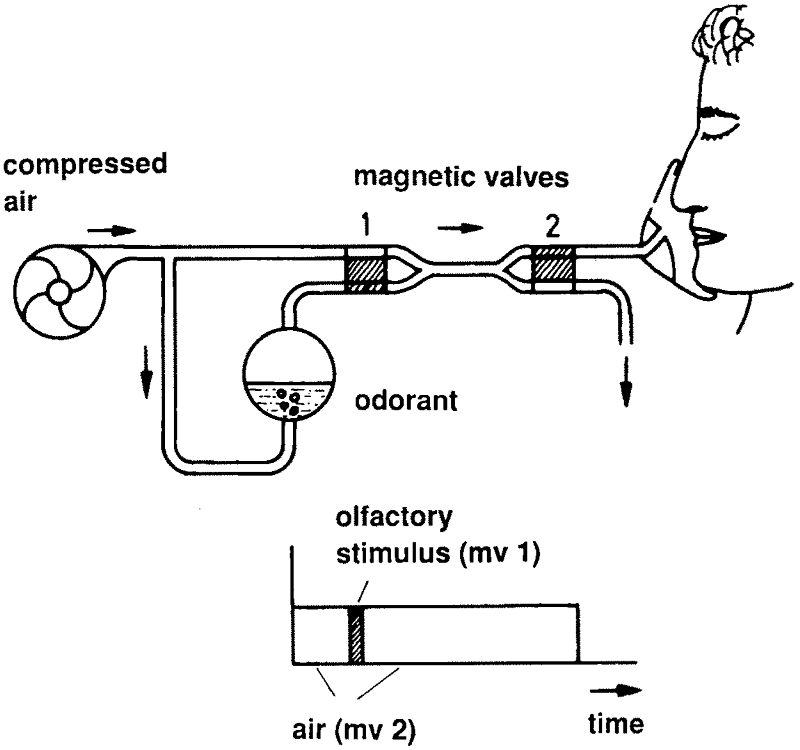

Fig 1. Principle of olfactory stimulation. Initiated by inhalation, flow of fresh air is directed into nostrils. Moment later it is bypassed for short time interval through odorant flask generating odor impulse without additional pressure or temperature changes. mv — magnetic valve.

a moderate intensity, easily discriminated by persons with normal olfaction. For a control, an odor-free water flask was mounted. Each of the three qualities was presented 30 times in random order. Thus, for the total of 90 inhalations, less than 10 minutes was required. The moderate intensity is achieved by blowing air for 80 milliseconds at 2.5L/min through a flask with pure lilac oil or saturated camphor solution in paraffin. Initiated by inhalation, the odor impulse is switched into the clean carrier air that streams for 3 seconds after the beginning of inhalation. Lower odor intensities are achieved by dilution. Examinations close to threshold were performed at a dilution of 1:64.

Electrical brain activity was recorded with centered electrodes attached to the vertex (positive) and one mastoid (reference). An isolated low-noise amplifier was used with a time constant of 2.5 seconds and an upper frequency limit of 15 Hz (24 dB per octave). Sweeps with a duration of 2.56 seconds (256 digitized values) were averaged into one of the three channels according to the three odor qualities. Sweeps that were too noisy, especially those due to motion artifacts, were rejected. The OEP and the CNV were simultaneously recorded from 11 patients with hyposmia, 12 with anosmia, and 6 with parosmia, and from a normal group of 10 persons with a similar age distribution. Grouping was performed according to the results of usual subjective olfactometry, and rhinomanometry proved normal respiration in all cases regarded here.

The OEP was expected between 0.6 and 0.9 second after stimulus onset (including the conveyance lag). In this time interval, the difference between maximal positive and negative deflections was taken for the OEP amplitude even where the typical OEP shape was missing. In such a case the readout may represent spontaneous activity. The CNV amplitude was defined as the deviation of a regression line between 1.0 and 1.7 seconds, extrapolated to the end of this time interval. Because of this formal procedure, the CNV amplitude may turn out negative or positive. Amplitude differences were statistically tested with a double-sided *t* test, and a significance level was chosen at 95% probability.

RESULTS

In order to demonstrate the combined evaluation of OEP and CNV recordings, a typical case is selected from each group, first for normal olfaction (Fig 2A). Data collection is triggered by the patient's inhalation simultaneously with the valve switching from fresh air to odor. After a conveyance time of 150 milliseconds through the short tubes, the substance enters the patient's nose. If the odor is lilac, a tone is presented beginning at 1.8 seconds. It is to be terminated as fast as possible by manual reaction. Camphor and the void stimulus condition (water) are not followed by a confirmatory tone. As the patient expects the tone after recognizing "lilac," a CNV can be observed as a negative-going slope developing in the average curve between 1.1 and 1.8 seconds. An

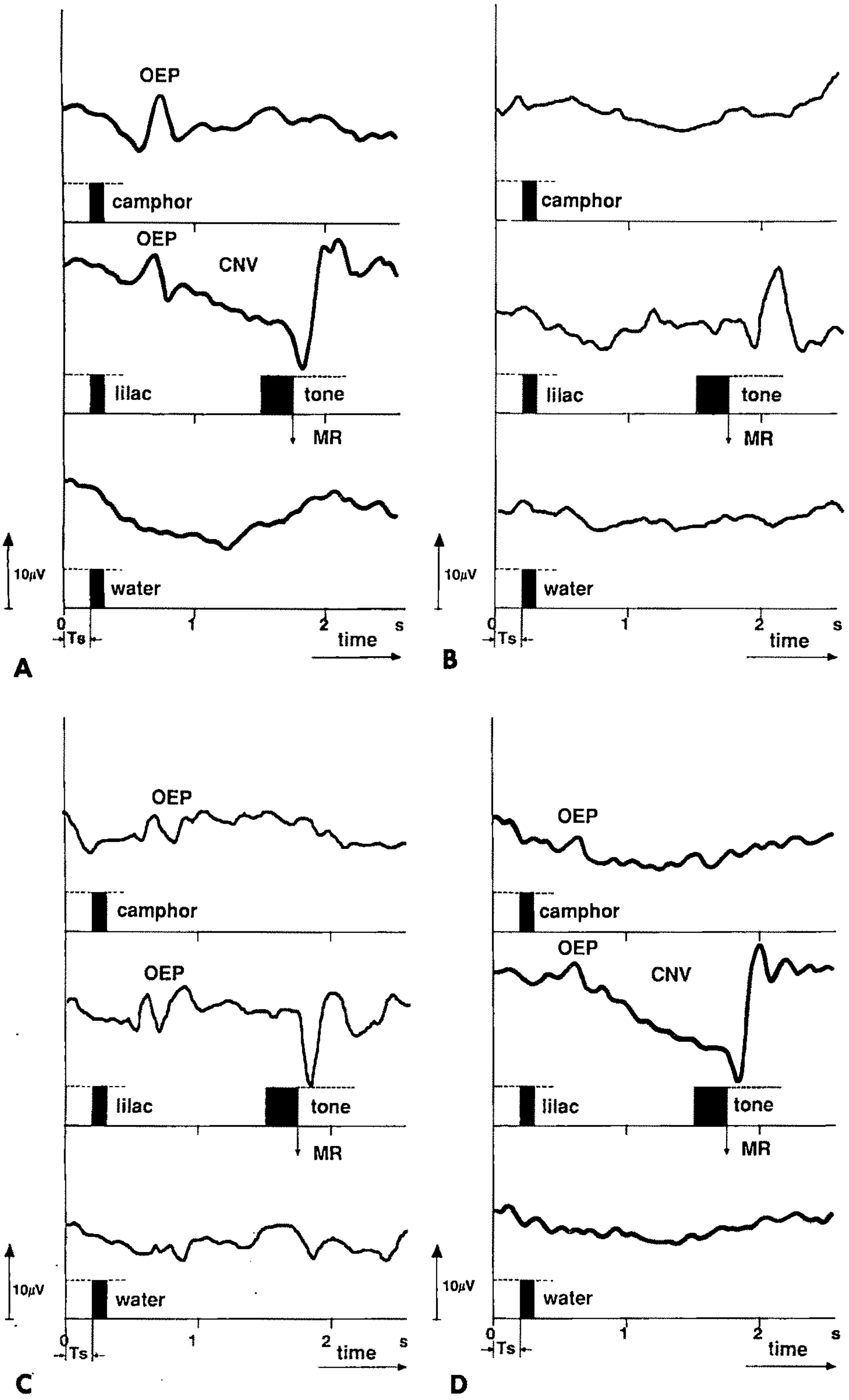

Fig 2. Typical cases. Ts — transport time of odorant. A) Normal olfaction. Olfactory evoked potential (OEP) and contingent negative variation (CNV) recorded simultaneously. OEP is evoked by "camphor" and "lilac" stimuli. CNV develops after "lilac" only while second, confirmatory stimulus is expected. Latter is to be terminated by manual reaction (MR). Neither OEP nor CNV is observed for "water" as control stimulation. B) Anosmia (no odor sensation). Neither OEP nor CNV is recorded. Confirmatory tone presented after "lilac" only evokes auditory response. C) Parosmia (no odor discrimination). OEPs are recorded for either odorant, but CNV is missing because patient does not know when to expect tone. D) Hyposmia (reduced olfactory sensitivity). Small OEPs are observed for either smell, together with enhanced CNV for "lilac."

OEP is found for both camphor and lilac, between 0.5 and 1 second, and neither OEP nor CNV can be seen in the "water" trace at the bottom. The residual low-frequency component may be due to the long time constant, and possibly reflects some uncertainty of expectation over a series of inhalations.

In a case of anosmia due to craniocerebral trauma (Fig 2B), neither an OEP nor a CNV can be obtained. Only an auditory response to the confirmatory acoustic stimulus after presentation of "lilac" can be observed. Patients with anosmia usually show some delay in their manual reaction because they are not warned through odor discrimination, so they can only respond to the tone.

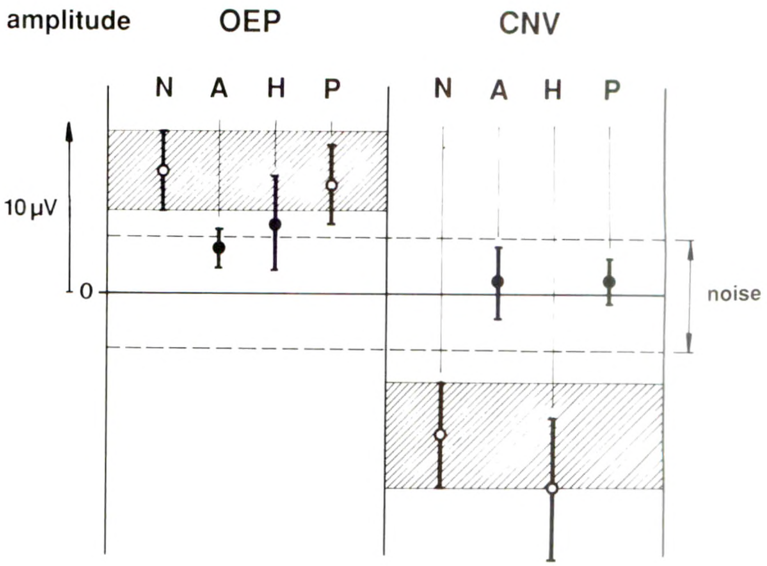

Fig 3. Mean amplitudes and standard deviations for olfactory evoked potentials (OEPs) and contingent negative variation (CNV). Anosmia (A, 12 cases), hyposmia (H, 11 cases), and parosmia (P, 6 cases) are compared to normal olfaction (N, 10 cases, hatched area). OEPs are reduced significantly with anosmia and hyposmia. No CNV is observed with anosmia or parosmia, and CNV is enhanced with hyposmia.

With parosmia as a result of rhinitis (Fig 2C), OEPs are present for either smell, but no CNV developed because the patient was unable to differentiate the two odors. As he accurately terminated the tone presented after lilac, we can assume he was attentive and really could not be sure when to expect "lilac." By the way, a different pattern could be observed in some patients with anosmia: a CNV of similar, although low amplitude in either smell trace as an indication of uncertainty or random expectation.

In a typical case of hyposmia (Fig 2D), OEPs are reduced for either odor. The CNV, however, shows a remarkably high amplitude. The patient said she used some effort to make out the smell and had to pay much attention to the discrimination task.

The amplitude evaluation of OEP and CNV was performed for a number of cases corresponding to the preceding examples (Fig 3). The mean amplitude values are shown together with their standard deviations. The OEP amplitudes on the left can only be positive because of the peak-to-peak readout. The CNV amplitudes on the right should be negative, but can as well turn out positive because of their reference to the baseline. Compared to normal olfaction, OEP amplitudes are significantly reduced for anosmia and hyposmia, while they are nearly normal for parosmia. The CNV amplitude is significantly reduced for anosmia and, as the patient is unable to differentiate the two odors, also for parosmia. In either case, they cannot be separated from spontaneous activity. In cases of hyposmia, however, the CNV amplitude is enhanced, although not significantly, probably because of the large variation in the degree of hyposmia.

A more reproducible experiment to verify enhanced CNV amplitudes for close-to-threshold stimuli was performed. A person with normal olfaction received olfactory stimuli of normal and very low, ie, just perceptible, intensity. The CNV recordings only (for lilac and the confirmatory tone) are presented in Fig 4. At a normal intensity (upper trace) the OEP and CNV amplitudes are similar to those of Fig 2A. Near threshold (bottom) the OEP is missing and the CNV amplitude is nearly doubled. This result was significant in the *t* test for matched pairs in a group of 10 adults with normal olfaction.

DISCUSSION

According to our results, differential diagnosis of

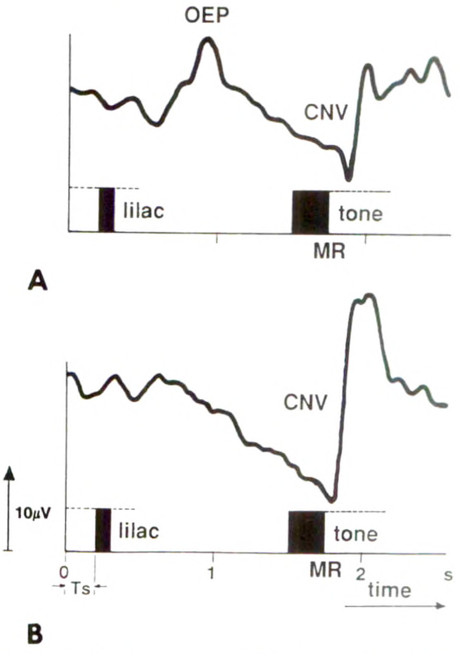

Fig 4. Stimulus intensity. Reactions to "lilac" only are shown for A) normal and B) low intensity. Close to perception threshold, OEP is vanishing, while CNV amplitude is nearly doubled. Abbreviations as in Fig 2.

normal olfaction, anosmia, parosmia, and hyposmia can be performed by simultaneous recording of OEP and CNV in a reasonable amount of time. A clearly detectable OEP proves odor perception, while the CNV ascertains odor discrimination. The cause of a missing OEP, however, need not be anosmia. We know from audiometry that cortical evoked potentials depend on vigilance and they may be canceled with changing vigilance or uneasiness. In olfactometry, conditions are still more difficult, because of the patient's upright position and the motoric activity required, ie, forced inhalation and pressing the button. On the other hand, the achievable number of repetitions is reduced by the patient's limited tolerance and by possible adaptation. With missing OEPs, the CNV has proved to be useful even for rejecting the diagnosis of anosmia. The main application of CNV, of course, is to diagnose parosmia. Very uneasy patients, however, can render both OEP and CNV inaccurate. Lack of attention only affects CNV, because no expectation develops if someone only responds to the confirmatory stimulus.

The enhanced CNV amplitude at low intensity of the primary stimulus was also reported by Prevec and Ribaric[12] for auditory signals. Their explanation is that very low stimuli activate the person's concentration. According to Rebert et al,[13] also, a lower confirmatory stimulus could enhance CNV, and the same occurred when the manual reaction was combined with some physical effort. Additional actions may increase the patient's attention, but can also make the task too complicated (manuscript in preparation).

The enhanced CNV amplitude of low odor intensity enables a special application of combined OEP and CNV recording: the quantification of discrimination threshold. Although OEP amplitudes are correlated with odor intensity in a group comparison,[9] single-case judgment of odor perception threshold may be difficult because of the large variation of OEP amplitudes for low stimuli. The CNV, however, is actually enhanced just above discrimination threshold, and rapidly vanishes when the intensity becomes still lower. This threshold may be quantified in a dilution series. Because of the large amount of time required, this procedure is only applicable with tolerant patients. For routine clinical diagnosis we have chosen one moderate odor intensity. We assume an anosmia or severe hyposmia if neither CNV nor OEPs are present, a parosmia if CNV is missing while OEPs are clearly present, and hyposmia if there is a high CNV with reduced OEPs. The degree of hyposmia is cautiously determined by OEP amplitude evaluation.

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KIKUCHI-FUJIMOTO DISEASE OF THE NECK

UPDATE

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Kikuchi-Fujimoto disease (KFD) is a rare entity of uncertain cause that commonly presents with persistently enlarged cervical lymph nodes unresponsive to antibiotic therapy. Although it usually follows a benign course, KFD has been repeatedly misdiagnosed as malignant lymphoma; hence, clinicians and pathologists alike need to be aware of this disease entity. The newest developments regarding the etiology and course of KFD are presented through a review of the literature and a recent illustrative case.

KEY WORDS — histiocytosis, lymphadenitis, necrosis, syndrome.

Kikuchi-Fujimoto disease (KFD), formerly known as subacute necrotizing histiocytic lymphadenitis, is an increasingly recognized cause of cervical lymphadenitis, well known in Japan since its initial description there in 1972.[1,2] Practically all reports on this disease entity have appeared in the pathology literature. To our knowledge, only one publication has appeared previously in the otolaryngologic literature.[3] More recently, the largest patient series has appeared,[4] and new developments regarding the etiology and course of this disease have been reported. Because of the increasing number of cases being reported, the otolaryngologist–head and neck surgeon must be aware of the manifestations of this disease, its clinical course, and the potential for confusion with malignant lymphoma.

This disease entity manifests clinically with localized cervical adenopathy, occasionally tender to palpation. The disease preferentially affects women in a 4:1 ratio. Although there is a wide age range, the majority of patients are under the age of 30 years. Fevers and chills usually occur in conjunction with upper respiratory tract symptoms. Less often, patients may complain of weight loss, nausea, vomiting, and night sweats. Approximately 50% of patients will have mild granulocytopenia; 70% will have erythrocyte sedimentation rates above 60 mm/h.[4-6]

Although KFD most commonly affects cervical lymph nodes, any nodal chain in the body can be involved. Patients have also presented with generalized lymphadenopathy and hepatosplenomegaly. Persistent adenopathy invariably requires an excisional biopsy, which subsequently establishes the presence of KFD. The disease runs a benign course, and the lymphadenopathy appears to resolve spontaneously after 4 to 6 months.[4,6]

The diagnosis of KFD can only be established histopathologically from biopsy specimens. The morphological hallmarks of this disease have been described by numerous authors in recent years.[4,5,7] Although the microscopic appearance of nodes affected by KFD can be variable, the principal histopathologic features can be summarized as follows: 1) patchy, circumscribed areas of eosinophilic material consistent with a necrotizing process, 2) significant karyorrhexis with fragments of nuclear debris ("nuclear dust") distributed in an irregular fashion throughout the areas of necrosis, 3) absence of granulocytes and paucity of plasma cells, 4) presence of transformed lymphocytes (immunoblasts), predominantly of T-cell origin, and 5) presence of so-called plasmacytoid T cells, or plasmacytoid monocytes.

Histopathologically, the degree of necrosis within individual nodes covers a broad range, and when it is scant, a misdiagnosis of malignant lymphoma can be made.[5] Indeed, after the descriptions of Kikuchi and Fujimoto, reviews in this country of specimens originally diagnosed as lymphoma revealed, not infrequently, cases that appear to be more consistent with the diagnosis of KFD.[5,6]

CASE REPORT

A 21-year-old man with no significant past medi-

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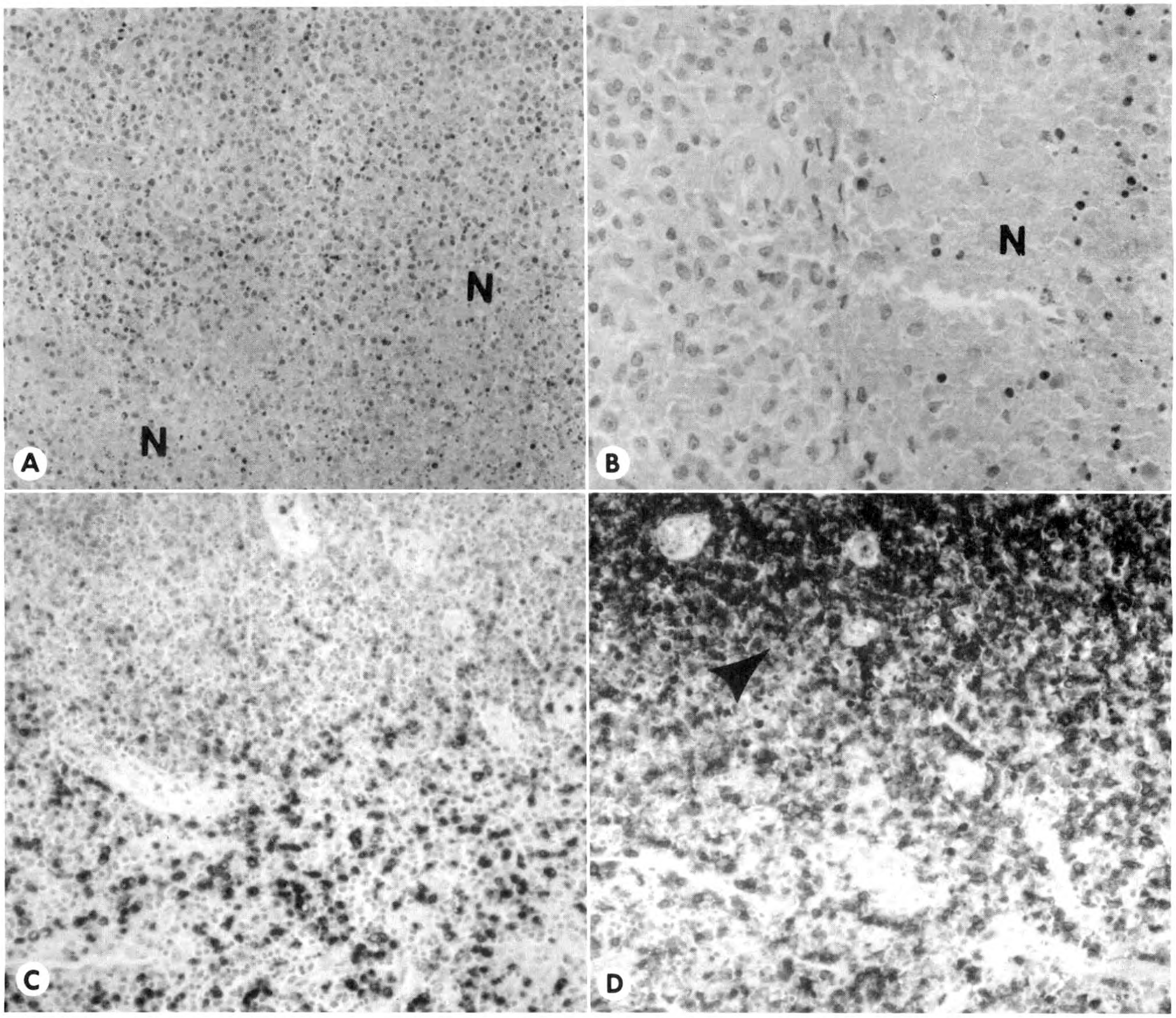

Lymph node exhibiting typical features of Kikuchi-Fujimoto disease. A) Low- and B) high-magnification views of partially effaced cervical lymph node (H & E, original x120 and x400, respectively). Area of necrosis (N), with abundant karyorrhectic debris, is surrounded by aggregates of histiocytes, plasmacytoid monocytes, and transformed lymphocytes (immunoblasts), with conspicuous absence of neutrophils, eosinophils, and plasma cells. C) Immunoperoxidase staining of same area of lymph node with KP-1/CD68 (monocyte-macrophage marker), demonstrating presence of numerous dark-staining cells of this lineage (H & E, original x120). D) Immunoperoxidase staining of same area with UCHL-1/CD45RO (marker of T-lymphocytes), demonstrating presence of numerous dark-staining cells of this lineage (H & E, original x120). Necrotic and karyorrhectic material (arrowhead) is cross-reactive for this antibody, resulting in high background staining in central necrotic areas of lesion.

cal history was referred to the otolarynology service complaining of a 2-week history of a slowly growing, painful right-sided neck mass. He had been experiencing daily fevers to 39°C, associated with night sweats, and had been feeling noticeably fatigued. The patient denied having a sore throat or other upper respiratory tract symptoms. His adenopathy had failed to respond to full courses of oral clindamycin and amoxicillin-clavulanate therapy. He denied weight loss, diarrhea, rashes, or abdominal discomfort.

Physical examination revealed a well-appearing male patient with a temperature of 38.5°C and a hard, moderately tender 3 x 3-cm mass in the right supraclavicular area, just behind the posterior border of the sternocleidomastoid muscle. In addition, there were multiple smaller nontender nodes in the posterior triangle of the right side of the neck. No other nodal chains were affected. Laboratory studies revealed a leukocyte count of 4,720/mm^3 with 67% neutrophils and 23% lymphocytes. The erythrocyte sedimentation rate was 75 mm/h. A chest radiograph was interpreted as normal. The patient was promptly admitted to the hospital for additional diagnostic studies.

A computed tomography (CT) scan revealed multiple 1-cm lymph nodes in the posterior part of the

SUMMARY OF REPORTED CASES OF KIKUCHI-FUJIMOTO DISEASE

		No. of Cases				
Authors	***Year***	***F***	***M***	***Mean Age***	***Site of Lymphadenopathy****	***Clinical Course***
Pileri et al[15]	1982	20	7	27	Cervical 15 Axillary 7 Supraclavicular 4 Other 1 Generalized 6†	Complete resolution 14 Not reported 13
Turner et al[10]	1983	27	3	28	Cervical 28 Axillary 4 Supraclavicular 2 Other 2	Complete resolution 21 Not reported 8 Recurrence 1
Evans et al[16]	1985	3		24	Cervical 3	Not reported 3
Gleeson et al[3]	1985	1		20	Cervical 1	Not reported 1
Chan and Saw[19]	1986	4	5	23	Cervical 7 Generalized 2	Complete resolution 7 Not reported 2
Unger et al[17]	1987	3	1	28	Cervical 3 Parotid 1	Complete resolution 2 Not reported 2
Rivano et al[18]	1987	8		28	Cervical 7 Supraclavicular 1	Complete resolution 8
Dorfman and Berry[4]	1988	85	23	30	Vast majority with cervical adenopathy, specific numbers not provided	Complete resolution 60 Not reported 44 Death after biopsy 1 Recurrence 3
Chan et al[14]	1989		1	38	Generalized; 11 nodal chains involved, including cervical nodes	Death after biopsy 1§
Chamulak et al[5]	1990	8	2	30	Cervical 7 Supraclavicular 3 Other 3	Complete resolution 7 Not reported 3
Total ψ	1982-1990	74	19	27	Cervical adenopathy 72 Supraclavicular 10 Axillary 12 Other 16	Complete resolution 59 Not reported 32 Recurrence 1 Death 1

*Numbers include all patients who presented with adenopathy at specified location. Several patients had involvement of two or (rarely) more nodal chains and were thus counted more than once.

†Number of cases with generalized lymphadenopathy that included cervical adenopathy was not specified.

§Same death as reported by Dorfman and Berry.[4]

ψExcludes patients from Dorfman and Berry,[4] since authors did not provide specific numbers.

neck. The right supraclavicular mass was measured at 1 cm on this study, although it was noticeably larger to palpation. A CT of the chest, abdomen, and pelvis revealed small left axillary nodes, with no further abnormalities. A fine-needle aspirate of the mass showed a nonspecific collection of inflammatory cells. Serology panels for Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, and *Toxoplasma* were negative; tuberculin purified protein derivative placement revealed anergy.

An excisional biopsy of the supraclavicular mass was performed without complications. Following the procedure, the fevers resolved, prompting discharge from the hospital. One month later the patient continued to do well, although his posterior neck adenopathy persisted. His leukocyte count on follow-up was 2,700/mm^3 with a normal differential. Complete resolution of the posterior neck adenopathy occurred after 4 months, and the patient continues to do well 17 months after his original presentation.

Histopathology. The excised neck mass consisted of a lymph node exhibiting the typical features of KFD (see Figure). Partial effacement of the nodal architecture was present in areas containing aggregates of histiocytes, plasmacytoid monocytes, and transformed lymphoid cells (immunoblasts), with abundant karyorrhectic debris, focal necrosis, and a conspicuous absence of neutrophils, eosinophils, and plasma cells. Focal areas of fibrinoid necrosis were also prominent. Immunoperoxidase studies performed on paraffin-embedded tissue sections revealed scattered as well as focal large aggregates of histiocytes and monocytes (KP-1, CD68-positive), particularly abundant within involved areas and associated with frequent UCHL-1/CD45RO–positive T-lymphocytes.[8,9] B-lymphocytes (L26/CD20-positive) occurred both in scattered fashion and as focal aggregates, mostly in residual areas of uninvolved lymph node. Granulomas were absent. Special stains for fungi and acid-fast pathogens, as well as immunoperoxidase stains for cytomegalovirus and herpes simplex virus, were negative for these organisms.

DISCUSSION

Kikuchi-Fujimoto disease remains largely unrecognized by otolaryngologists in Western countries as a cause of benign lymphadenopathy. This may be in part because of its presumed low incidence, although in recent years it has been realized that numerous cases previously diagnosed as malignant lymphoma may be more consistent with KFD. The true incidence of KFD may thus be higher than originally thought. Turner et al[10] reported on 30 cases of KFD, 40% of which had originally been diagnosed histopathologically as large cell lymphoma or Hodgkin's disease. Some of these patients had received active therapy for their presumed malignancy.

The relative rarity of KFD has permitted various aspects of this disease to remain confusing. First, the issue of naming this entity continues to present difficulties, as it is known by any one of multiple permutations of the term "subacute histiocytic necrotizing lymphadenitis." Recently, an attempt has been made to clarify matters by naming the disease after the authors who initially described it in Japan.[5]

Another point of confusion is the so-called plasmacytoid T cell, one of the critical morphological elements in establishing the diagnosis of KFD. Although these cells exhibit features of T cells (CD-4–positive), their immunoreactivity to a number of monocyte-macrophage markers establishes them as members of this latter lineage.[11,12] Chamulak et al[5] point out that at the light microscopic level, so-called plasmacytoid T cells bear no resemblance to plasma cells. They propose that pathologists unfamiliar with KFD may fail to correctly identify these cells when present (as in some of the erroneously diagnosed cases of malignant lymphoma), or may erroneously identify cells with true plasmacytoid features, such as transformed lymphocytes with abundant cytoplasm, as the plasmacytoid T cells characteristic of KFD.

The cause of KFD remains unclear. Initial reports linking KFD to acute toxoplasmosis on the basis of elevated serum titers in several patients have not been subsequently supported. A similar controversy surrounds the proposed roles of Epstein-Barr virus, parainfluenza virus, and *Yersinia enterocolitica* in the causation of KFD. Turner et al[10] pointed out that the absence of pharyngitis, the uniformly negative cultures, and the resolution of symptoms without antibiotics suggest a nonbacterial cause. In 1991, utilizing in situ hybridization techniques, Hoffman et al[13] demonstrated human herpesvirus 6 genomes in the excised lymph node of a patient with KFD. Ultrastructural studies demonstrating tubuloreticular structures in the cytoplasm of stimulated lymphocytes and histiocytes in KFD suggest a possible relation to hyperimmune lymphadenitis, in which tubuloreticular structures are numerous in endothelial cells, lymphocytes, and histiocytes. We agree with Chan et al,[14] who suggest that there is probably no one single cause for this disease, but rather that it may result from a hyperimmune reaction to various infectious, chemical, physical, and neoplastic agents.

To date, approximately 200 cases of KFD have been reported (see Table[3,4,5,10,14-19]). The predilection of this entity for cervical nodes is indeed striking; it affects these nearly 80% of the time. Within the neck, there is likewise a predilection for the posterior neck nodes. Turner et al[10] reported posterior neck involvement in 65% of patients with exclusively cervical KFD. Chamulak et al[5] reported 67% posterior neck involvement among patients with unifocal neck disease. The next most common sites of involvement are the axillary (14%) and supraclavicular (12%) nodal chains. Involvement of one or two nodal chains is the rule; involvement of three or more chains is unusual. Only 4 of 118 (3%) patients in whom there is follow-up have experienced a recurrence. Repeat biopsies in these cases have shown either nonspecific reactive changes or a recurrence of KFD.[10]

The present case illustrates various clinical and pathologic features of KFD. This patient had localized cervical adenopathy associated with fevers and night sweats, all of which were unresponsive to antibiotic therapy. Laboratory tests revealed a mild leukopenia, which is associated with this disease in 50% of cases, and a markedly elevated erythrocyte sedimentation rate, which likewise accompanies this disease in 70% of patients.[4-6] Other causes of localized lymphadenopathy were ruled out by the panel of serologic tests now routinely used to evaluate febrile adenopathy syndromes. True to the benign, self-limiting course of this disease, the patient was doing well on his last visit, approximately 17 months after his original presentation.

At present, there are no noninvasive tests that can be used to establish the diagnosis of KFD. This disease cannot be diagnosed on fine-needle aspiration. For this reason, open biopsy of lymph nodes that do not respond to appropriate antibiotic therapy is in order. Once a diagnosis of KFD has been established by biopsy, any nonanalgesic therapy should be discontinued, and the disease should be allowed to run its course.

All reports to date indicate that the adenopathy of

KFD spontaneously resolves within 6 months in practically all patients.[5,6,8] Of note, two patients have developed systematic lupus erythematosus after initially receiving diagnoses of KFD. For this reason periodic follow-up of patients with KFD for several years is recommended, and an appropriate lupus workup should be performed should other signs and symptoms of this entity appear.[4]

Fatalities associated with KFD are rare, if they occur at all. Chan et al[14] reported in 1989 on a 38-year-old man with splenomegaly and multifocal KFD involving at least 12 distinct nodal groups who died of cardiogenic shock 3 days after cervical lymph node biopsy under local anesthesia. This case is extremely unusual because of the high number of nodal chains involved. The authors propose that cardiac damage may have been mediated by cytokines secreted in large amounts by the histiocytes in the numerous affected lymph nodes. Dorfman and Berry[4] reported fatalities in five patients with KFD: one was the patient just described, and the other four patients died of unrelated causes. It is probably safe to assume that unless an extensive degree of multifocal disease is present, patients with KFD are assured of an excellent prognosis.

The differential diagnosis of a slowly growing neck mass is broad. An excellent summary of the differences in histopathologic findings among the most important causes of lymph node enlargement has been provided by Dorfman and Berry.[4] Given the marked predilection of this disease for cervical lymph nodes, the otolaryngologist–head and neck surgeon will play an important role in the diagnosis of KFD. The increasing number of reports of KFD should alert clinicians and pathologists to the existence of this disease entity. Knowledge of its clinical and histologic features is mandatory if serious errors in diagnosis and treatment are to be avoided.

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SUBSTANCE P AND VASOACTIVE INTESTINAL PEPTIDE IN NASAL SECRETIONS AND PLASMA FROM PATIENTS WITH NASAL ALLERGY

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To clarify the role of substance P (SP) and vasoactive intestinal peptide (VIP) in nasal allergy, we measured their concentrations in the nasal secretions and plasma of normal subjects and patients with nasal allergy to house dust and Japanese cedar pollen by competitive enzyme-linked immunoassay. The mean levels of SP (224 pmol/L) and VIP (41.6 pmol/L) in the nasal secretions of normal subjects were significantly higher than those in plasma (SP 3.04 pmol/L and VIP 1.04 pmol/L; $p < .01$). The mean levels of SP and VIP in the nasal secretions of the pollinosis group were significantly higher than those of the control group ($p < .05$ and $p < .01$), while the levels of the house dust allergy group were not higher than those of the control group. Intranasal allergen challenge significantly reduced SP levels in the nasal secretions of the allergy groups, while it did not influence VIP levels in the nasal secretions. These findings suggest that SP and VIP are actively secreted into the nose and may play an important role in the allergic reaction on the surface of the human nasal mucosa.

KEY WORDS — competitive enzyme-linked immunoassay, nasal allergy, nasal secretions, substance P, vasoactive intestinal peptide.

INTRODUCTION

Various neuropeptides, such as substance P (SP), calcitonin gene-related peptide, and vasoactive intestinal peptide (VIP), are present in the sensory and autonomic neurons in the human nasal mucosa.[1-3] They have various physiologic activities and act as chemical mediators as well as neurotransmitters.[3-6] Substance P neurons are observed in the afferent sensory branches of the trigeminal nerve, innervating the walls of the submucosal glands and blood vessels and the epithelium, and VIP coexists with acetylcholine in the parasympathetic neurons surrounding the submucosal glands and vessels. These neuropeptides are suspected to play an important role in allergic reaction of the nasal mucosa, not only as neurotransmitters but also through axon reflexes, neurogenic inflammation, and mast cell–nerve interaction.[7-9]

However, the precise role and action of the neuropeptides in allergic reactions of the nasal mucosa are still unclear. In an effort to elucidate the role of neuropeptides in nasal allergy, we measured concentrations of SP and VIP in nasal secretions and plasma from patients with nasal allergy and investigated the influence of intranasal provocation of allergic reactions on the levels of those neuropeptides in nasal secretions.

MATERIALS AND METHODS

Subjects. Nasal secretions and plasma samples were collected from patients with nasal allergy to house dust (HD) and Japanese cedar pollen and from healthy control subjects without allergy or other diseases. The concentration of SP was examined in the nasal secretions and plasma of 50 patients with nasal allergy (30 patients with HD allergy and 20 with pollinosis) and 30 controls. The VIP levels were examined in the same number of samples from other allergy patients and controls.

For SP analysis, the mean ages of the HD allergy, pollinosis, and control groups were 23 years (10 to 58 years), 36 years (15 to 62), and 37 years (14 to 69), respectively. The corresponding ages for VIP analysis were 25 years (12 to 60), 34 years (14 to 62), and 31 years (23 to 61).

Antisera. Anti–substance P (Cambridge Research Biochemicals Ltd, Cambridge, England) and anti-VIP antisera (UCB Bioproducts SA, Brussels, Belgium) of rabbit, and anti-rabbit immunoglobulin G (IgG) antiserum of goat (Boehringer Mannheim Corp, Mannheim, Germany, and TAGO Inc, Burlingame, Calif) were purchased from commercial sources.

Collection of Nasal Secretions and Plasma Samples. Nasal secretions were collected from patients with nasal allergy before and after nasal provocation by using Juhn Tym-Tap tubes, and 0.3 mL of aprotinin (500 kallikrein inhibiting units per milliliter) and

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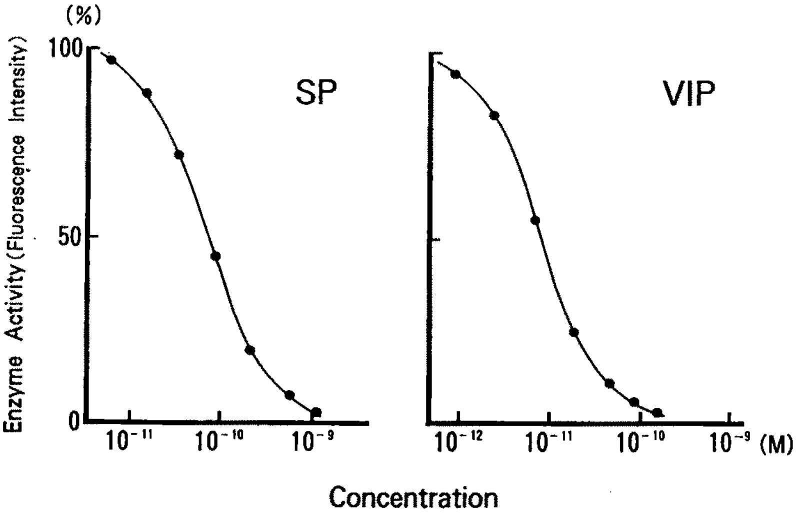

Standard curves of substance P (SP) and vasoactive intestinal peptide (VIP) as measured by competitive enzyme-linked immunoassay.

ethylenediaminetetraacetic acid (EDTA; 1.2 mg/mL) were immediately added to each tube. At the same time, 10 mL of venous blood was collected, mixed with 0.5 mL of the aprotinin-EDTA mixture, and centrifuged to produce plasma samples. Those samples were kept at –40°C until use.

An allergic reaction of the nasal mucosa was provided by applying a paper disc containing HD and Japanese cedar pollen allergens on the nasal mucosa of the inferior turbinate. Nasal secretions were collected 10 minutes after provocation. In the pollinosis group, nasal secretions were obtained from March to April, when Japanese cedar pollen is released into the air.

Elution of SP and VIP From Nasal Secretions and Plasma Samples. Substance P and VIP were eluted from the nasal secretions and plasma samples with a Sep-Pak C18 cartridge for immunochemical analysis according to the method previously described by Takeyama et al.[10,11]

Nasal secretions and plasma samples were diluted fivefold with 4% acetic acid and were applied to the Sep-Pak C18 cartridge. After washing with 10 mL of 4% acetic acid, SP was eluted with 0.5% acetic acid solution containing 70% acetonitrile. For VIP, samples were applied on the column after threefold dilution with 3% acetic acid. The VIP was eluted with the acetic acid–acetonitrile solution after washing with 10 mL of 3% acetic acid. These eluates were concentrated by spin-vacuum evaporation, lyophilized, and dissolved in 100 μL of 0.05 mol/L phosphate buffer containing 0.5% bovine serum albumin, 1 mmol/L magnesium chloride, and 250 kallikrein inhibiting units/mL aprotinin for the immunochemical analyses.

Measurement of SP and VIP Concentrations in Nasal Secretions and Plasma. The concentrations of SP and VIP in the nasal secretions and plasma were measured by competitive enzyme-linked immunoassay according to the method reported by Takeyama et al.[10,11]

One hundred microliters of anti-SP or anti-VIP rabbit antiserum was mixed with 100 μL of SP or VIP eluate and incubated at 4°C for 24 hours. Fifty microliters of SP or VIP labeled with β-D-galactosidase was added to these antigen-antibody mixtures and incubated at 4°C for 24 hours. Enzyme-labeled SP and VIP were prepared by the method of Kitagawa et al.[12] Then, 100 μL of the mixtures were applied to each well of a microtiter plate coated with anti-rabbit IgG goat antiserum and incubated overnight at 4°C. After four washings with a 0.05% Tween 20 solution of 0.01 mol/L phosphate-buffered saline at pH 7.2, 200 μL of a 0.1 mmol/L 4-methylumbelliferyl β-D-galactopyranoside solution in 0.05 mol/L phosphate buffer at pH 7.0 containing 1 mmol/L magnesium chloride as a substrate was added to each well of the plate and incubated at 37°C for 3 hours. The fluorescent intensity of each well was measured at 360 nm of the exciting beam.

Measurement of Total Protein Concentration in Nasal Secretions. The total protein concentration in the nasal secretions was determined by the method of Lowry et al.[13]

RESULTS

Standard Curves of SP and VIP. The Figure shows the standard curves of the SP and VIP concentrations measured by competitive enzyme-linked immunoassay. With this immunochemical analysis, the measurable range of SP concentration was from 6 to 300 pmol/L (0.24 to 12 fmol per well), and that of VIP was from 1 to 40 pmol/L (0.04 to 1.6 fmol per well).

SP Concentrations in Nasal Secretions and Plas-

TABLE 1. CONCENTRATIONS OF SUBSTANCE P IN NASAL SECRETIONS AND PLASMA

			Nasal Secretions		
		Units	*Before Provocation*	*After Provocation*	*Plasma*
House dust allergy (n = 30)	SP	pmol/L	274 ± 190^{a}	179 ± 118^{j}	3.64 ± 1.85^{p}
		pg/mL	438 ± 304	286 ± 189	5.38 ± 2.96
	TP	mg/mL	4.94 ± 1.97^{b}	4.11 ± 1.37^{k}	
	SP/TP	x10^{-8}	8.82 ± 4.03^{c}	6.87 ± 3.18^{l}	
Pollinosis (n = 20)	SP	pmol/L	315 ± 126^{d}	181 ± 69^{m}	3.32 ± 1.25^{q}
		pg/mL	504 ± 202	290 ± 110	5.31 ± 2.00
	TP	mg/mL	6.26 ± 3.03^{e}	4.96 ± 1.33^{n}	
	SP/TP	x10^{-8}	8.85 ± 3.23^{f}	6.11 ± 2.50^{o}	
Controls (n = 30)	SP	pmol/L	224 ± 116^{g}		3.04 ± 1.05^{r}
		pg/mL	358 ± 186		4.86 ± 1.68
	TP	mg/mL	7.52 ± 3.22^{h}		
	SP/TP	x10^{-8}	5.16 ± 2.42^{i}		

Data are means ± SD.

SP — substance P, TP — total protein.

a > j, d > m, c > i, f > i, h > b, a > p, d > q, and g > r at p < .01.

c > l, e > n, f > o; and d > g at p < .05.

ma. The mean concentrations of SP in the nasal secretions and plasma of each group are exhibited in Table 1.

In the control group, the mean concentration of SP was 224 pmol/L (358 pg/mL) in the nasal secretions and 3.04 pmol/L (4.86 pg/mL) in plasma. The mean SP concentration in the nasal secretions was significantly higher than that in plasma (p < .01).

In the allergy groups, the mean concentration of SP in the nasal secretions was 274 pmol/L (438 pg/mL) for the patients with HD allergy and 315 pmol/L (504 pg/mL) for those with pollinosis before intranasal provocation of the allergic reaction. These values were significantly higher than those of plasma (p < .01). Although the mean concentrations of SP in plasma were not significantly different among the HD allergy, pollinosis, and control groups (p > .05), the mean level of SP in the nasal secretions of patients with pollinosis was significantly higher than that of the control group (p < .05). The mean ratio of SP to total protein in the nasal secretions of the allergy groups was 8.82 x 10^{-8} for HD allergy and 8.85 x 10^{-8} for pollinosis. These values were significantly higher than that of the control group (5.16 x 10^{-8}; p < .01), although they were not significantly different from each other (p > .05).

After intranasal provocation of the allergic reaction, the SP concentration and the SP to total protein ratio in the nasal secretions of the HD allergy and

TABLE 2. CONCENTRATIONS OF VASOACTIVE INTESTINAL PEPTIDE IN NASAL SECRETIONS AND PLASMA

			Nasal Secretions		
		Units	*Before Provocation*	*After Provocation*	*Plasma*
House dust allergy (n = 30)	VIP	pmol/L	39.0 ± 28.8^{a}	28.6 ± 28.4	1.03 ± 0.92^{j}
		pg/mL	156 ± 115	114 ± 114	4.12 ± 3.68
	TP	mg/mL	5.73 ± 2.23^{b}	4.45 ± 1.54	
	VIP/TP	x10^{-8}	2.89 ± 2.03^{c}	2.34 ± 1.72	
Pollinosis (n = 20)	VIP	pmol/L	82.9 ± 61.3^{d}	77.2 ± 11.8	1.81 ± 2.31^{k}
		pg/mL	332 ± 245	308 ± 47.2	7.24 ± 9.24
	TP	mg/mL	5.95 ± 2.23^{e}	5.24 ± 1.48	
	VIP/TP	x10^{-8}	6.50 ± 6.33^{f}	5.27 ± 7.36	
Controls (n = 30)	VIP	pmol/L	41.6 ± 34.0^{g}		1.04 ± 0.49^{l}
		pg/mL	166 ± 136		4.16 ± 1.96
	TP	mg/mL	8.34 ± 2.77^{h}		
	VIP/TP	x10^{-8}	2.05 ± 1.35^{i}		

Data are means ± SD.

VIP — vasoactive intestinal peptide, TP — total protein.

a > j, d > k, and g > l at p < .01.

d > a, d > g, f > c, f > i, h > b, and h > e at p < .05.

pollinosis groups significantly decreased in comparison with the values observed before provocation ($p < .01$).

VIP Concentrations in Nasal Secretions and Plasma. Table 2 shows the mean concentration of VIP in the nasal secretions and plasma of each group.

In the control group, the mean concentration of VIP was 41.6 pmol/L (166 pg/mL) in the nasal secretions and 1.04 pmol/L (4.16 pg/mL) in plasma. The mean VIP concentration in the nasal secretions was significantly higher than that of plasma ($p < .01$).

In the allergy groups, the mean concentration of VIP in the nasal secretions was 39.0 pmol/L (156 pg/mL) for the HD allergy group and 82.9 pmol/L (332 pg/mL) for the pollinosis group before intranasal provocation. These values were significantly higher than those of plasma ($p < .01$). The mean concentration of VIP in the nasal secretions of patients with pollinosis was significantly higher than those of the HD allergy and control groups ($p < .05$), while the mean VIP concentration in the nasal secretions was not significantly different between the HD allergy and control groups ($p > .05$). The mean ratio of VIP to total protein in the nasal secretions of the pollinosis group (6.50×10^{-8}) was also significantly higher than those of the HD allergy and control groups (respectively, 2.89×10^{-8} and 2.05×10^{-8}; $p < .05$).

After intranasal provocation, the mean VIP concentration and the VIP to total protein ratio of the allergy group were decreased, but these reductions of VIP in the nasal secretions were not significant ($p > .05$).

DISCUSSION

Substance P is present in the afferent sensory neurons of the trigeminal nerve on the human nasal mucosa, and VIP coexists with acetylcholine in the parasympathetic neurons. However, the precise role of these neuropeptides in allergic reactions on the nasal mucosa is still unclear. Allergic reactions on the nasal mucosa are associated with histamine and other chemical mediators. Histamine is released from mast cells and produces various symptoms such as hypersecretion, nasal blockage, and sneezing through nervous reflexes between the sensory and autonomic nerves. Histamine depolarizes sensory nerves possessing histamine receptors, and the neuronal wave of depolarization is centrally conveyed to the secretovasomotor and sneezing centers of the brain. From these centers, nerve impulses transmitted to autonomic nerves innervating the walls of the blood vessels and submucosal glands induce the secretion of various neurotransmitters from nerve endings. These neurotransmitters cause vasodilation and increased permeability of blood vessels and hypersecretion of submucosal glands and produce various symptoms in allergic reactions. Hypersecretion on the human nasal mucosa is mainly elicited by nervous reflexes engaging sensory neurons of the trigeminal and parasympathetic nerves.[14,15]

In addition to nervous reflexes, axon reflexes, neurogenic inflammation, and mast cell–nerve interactions are also thought to play important roles in allergic reactions and to be mediated by various neuropeptides.[7-9] Small-diameter and unmyelinated fibers of the sensory neurons are extensively branched, densely innervate the walls of submucosal vessels and glandular acini, and form neurosecretory varicosities in the vascular and glandular regions. The neuronal wave in the sensory neurons evoked by histamine immediately extends to the extensively branched system of the peripheral sensory neurons, as well as centrally to the brain. As a result, various neuropeptides are released from the nerve endings of the sensory neurons into spaces near the submucosal vessels and glands and elicit the rapidly deployable reactions of the submucosal vessel and gland. Furthermore, mast cells are frequently observed close to the SP neurons of the sensory nerve,[9] and high doses of SP induce histamine release from mast cells.[16]

In the present study, we determined the baseline levels of SP and VIP in the nasal secretions and plasma of normal subjects. The mean concentration of SP in the nasal secretions of the control subjects without nasal allergy or other diseases was 224 pmol/L (358 pg/mL) and about 70 times higher than that of plasma (3.04 pmol/L, 4.86 pg/mL). The mean level of VIP in the nasal secretions was 41.6 pmol/L (166 pg/mL) and about 40 times higher than that of plasma (1.04 pmol/L, 4.16 pg/mL). Tønnesen and Schaffalitzky de Muckadell[17] previously measured the concentrations of SP and VIP in serotonin-induced nasal secretions of normal subjects by radioimmunoassay. Intranasal serotonin challenge induces hypersecretion of the submucosal gland and may cause dilution of the nasal secretions. Their values for SP and VIP concentrations in serotonin-induced nasal secretions were lower than the levels reported in the present study. Therefore, our results may reflect more precise levels of SP and VIP in nasal secretions because we collected and used nasal secretions from normal subjects without any intranasal stimulation to induce hypersecretion of the nasal gland. Although different methods to quantify the concentrations of SP and VIP were employed in previous studies, the SP and VIP concentrations in the nasal secretions were higher than those in plasma. This finding is consistent with our results and suggests that SP and VIP are actively secreted into the nasal secretions from the nerve endings of the nasal mucosa.

The SP concentrations in the nasal secretions of patients with pollinosis were significantly higher than those of control subjects, while SP levels in the nasal secretions of patients with HD allergy were not higher than those of the control group. However, the SP–total protein ratios in the nasal secretions of patients with HD allergy and pollinosis were significantly higher than those of normal subjects. In contrast to SP, VIP concentrations and VIP to total protein ratios in the nasal secretions of patients with pollinosis were significantly higher than those of normal subjects, while neither parameter in the nasal secretions was significantly different between the HD allergy and control groups. The disparity between SP and VIP concentrations in the nasal secretions of patients with nasal allergy may be attributed to the differences in immunochemical and biochemical characteristics of the allergens and responses of patients with nasal allergy to the allergens.

Tønnesen et al[18] showed that intranasal allergen challenge had no effect on the concentrations of serotonin and SP in the nasal secretions of patients with hay fever, while it induced an increase of VIP levels in plasma. Also, Walker et al[19] could not find any differences in SP concentrations in the nasal secretions between normal subjects and patients with rye grass allergy, and they concluded that intranasal allergen challenge did not have any effect on the SP levels in the nasal secretions. They suggested that the lack of increase of the SP levels in the nasal secretions after intranasal allergen challenge is consistent with the fact that nasal aerosols of SP have only a small effect on nasal airway resistance and evoke no allergic symptoms. However, in the present study, SP levels in the nasal secretions of patients with nasal allergy to HD and Japanese cedar pollen were significantly decreased by intranasal allergen challenge, while VIP levels were not influenced by intranasal stimulation by the antigens. The discrepancy between the effects of intranasal allergen challenge on SP and VIP levels in the nasal secretions may depend on the state of the nasal mucosa of the patients with nasal allergy. We collected nasal secretions from patients with nasal allergy when they were suffering from allergic symptoms such as sneezing, nasal hypersecretion, and nasal obstruction. Therefore, the nasal mucosa of these patients was already stimulated and hypersensitive to the allergen. Because of the continuous stimulation by the allergens, the active secretion of SP from the nasal mucosa was ongoing, and its level in the nasal secretions was already elevated. Under these conditions, intranasal allergen challenge may induce a temporal exhaustion of SP in the nasal mucosa and decreased SP concentrations in the nasal secretions.

Although many studies[3-6, 20,21] have demonstrated various activities of neuropeptides on the blood vessels and submucosal glands of the airway mucosa, the physiologic effects of SP and VIP on the surface of the human nasal mucosa are still controversial. The topical application of SP to the human nasal mucosa causes no clinical symptoms such as sneezing, nasal secretion, or nasal obstruction, and has no effect on mucociliary transport or local histamine release. However, SP induces various responses of the airway mucosa in other species such as rats, dogs, and rabbits.[4-6,20] Vasoactive intestinal peptide also fails to produce clinical symptoms of nasal allergy, while this neuropeptide stimulates histamine release from mucosal mast cells of rats[21] and lactoferrin release from serous acinar cells of human nasal mucosa.[3] Khalil et al[21] suggested that VIP failed to cause airway responses because the vasodilator activity of VIP modulated a neurogenic inflammatory response induced by SP. The failure of SP and VIP to evoke clinical symptoms of nasal allergy in humans may be attributed either to the rapid enzymatic degradation of the peptides in the nasal secretions or to differences in sensitivity to the neuropeptides among various species. In order to define the precise activity and roles of SP and VIP on the surface of the human nasal mucosa, more studies are needed to clarify the immunochemical and biochemical characteristics of these neuropeptides.

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1993 CONFERENCE ON IMPLANTABLE AUDITORY PROSTHESES

The 1993 Conference on Implantable Auditory Prostheses will be held July 11-15, 1993, on the campus of Bryant College in Smithfield, Rhode Island. This is the sixth in a series of biennial research conferences originating with the 1983 Gordon Conference. The meeting will provide a forum for close interaction among scientists in all disciplines relating to the field of cochlear implants. For further information, contact Chris van den Honert, Division of Otolaryngology–Head & Neck Surgery, Box 3550, Duke University Medical Center, Durham, NC 27710; Telephone: (919) 684-6099, E-Mail: chx@dukehrl.mc.duke.edu.

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EXTRACTION OF INNER EAR ANTIGENS FOR STUDIES IN INNER EAR AUTOIMMUNITY

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The search for a diagnostic assay in patients with autoimmune inner ear disease has led to the preparation of antigens from the inner ear, a presumed target in this disorder. In order to standardize the antigen preparations currently being used in the Western blot immunoassay, we have examined several distinct extraction procedures that employ well-known solutions and detergents. Results of this investigation clearly show that antigens of interest (68 kd, 33 to 35 kd, and 32 kd) are optimally extracted with a detergent (0.5% sodium dodecyl sulfate) and that bovine serum albumin, a potential contaminant, can be removed in the water-soluble fractions. Purification of inner ear antigens by these methods will lead to more reproducible results in immunoblotting, as well as a greater opportunity to identify the mechanisms involved in autoimmune inner ear disease.

KEY WORDS — autoimmune inner ear disease, inner ear antigen.

INTRODUCTION

In previous reports, we have shown that a crude extract from bovine inner ear (IE) tissue is useful as an antigen source for screening autoantibodies against the IE in patients with rapidly progressive sensorineural hearing loss by Western blotting[1,2] and for the induction of experimental autoimmune labyrinthitis in guinea pigs.[3,4] Despite some progress, research in this area has been limited by the unavailability of highly purified antigen preparations. Having only a crude antigen preparation is disadvantageous for several reasons. One is that the antigenic epitopes involved in autoimmune inner ear disease (AIED) may be in low concentrations in the crude antigen preparation, so that immunoassays become less sensitive, often because of competition of the patient's serum with multiple antigenic targets. Another is that use of this antigen mixture during immunizations for the development of experimental AIED models will inevitably result in antibodies against several antigenic determinants, creating a condition that may not accurately reflect the human situation.

Therefore, the purpose of this study was to evaluate the purification schemes available for the extraction of IE antigen in order to facilitate the diagnostic sensitivity of immunoblotting in patients with autoimmune sensorineural hearing loss.

METHODS AND MATERIALS

Patients. Sera from 140 patients with progressive idiopathic sensorineural hearing loss, stored frozen at –70°C at the University of California, San Diego, Medical Center were screened at 1:1,000 dilution by the Western blotting technique previously reported by our laboratory.[1] Sera from 3 patients that showed typical and strongly positive patterns for the crude IE extracts were selected for further study. Sera from 10 normal individuals were negative at a dilution of 1:1,000 for these same tissue extracts.

Antigen. Fresh bovine temporal bones were obtained from an abattoir, frozen at –20°C, and stored until ready for dissection. The cochlear and vestibular membranous labyrinthine tissues and modiolus were removed from temporal bones under microscopy, place in chilled 50 mmol/L Tris buffer containing 1 μg/mL aprotinin, pH 6.8, and frozen at –70°C until used. The IE tissues removed from 10 temporal bones were thawed, teased apart,and homogenized by using a Plotter-Elvehjem tissue grinder in 10 mL of the aforementioned buffer, and then centrifuged at 100,000*g* for 1 hour at 4°C. The supernatant was saved as the "water-soluble" fraction, and the pellet was removed for further processing. The pellets were extracted in 50 mmol/L Tris buffer, pH 6.8, containing 2 mmol/L phenylmethylsulfonyl fluoride, 4 mmol/L ethylenediaminetetraacetic acid, and 10 mmol/L *N*-ethylmaleimide with one of the following solutions or detergents: 1) 0.15 mol/L sodium chloride (NaCl), 2) 0.5 mol/L NaCl, 3) 1% 3-[(cholamidopropyl)-dimethyl ammonio]-1-propanesulfonate

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This research was presented at the Fourteenth Midwinter Research Meeting of the Association for Research in Otolaryngology, St Petersburg, Florida, November 8-10, 1990.

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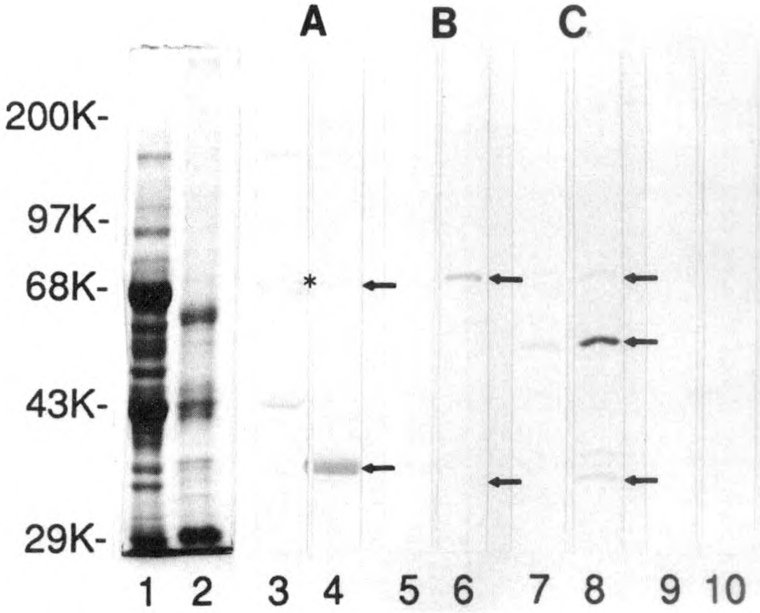

Fig 1. Protein patterns of water-soluble and sodium dodecyl sulfate (SDS)–soluble fractions and immunoblots of sera from patients A through C against fractions. Lanes 1, 3, 5, and 7 are water-soluble fractions, and lanes 2, 4, 6, and 8 are SDS-soluble fractions. Lanes 1 and 2 had Coomassie blue staining. Lanes 3 and 4 were incubated with patient A serum. They show 33 to 35 kd inner ear (IE)–specific binding and 62 kd binding (arrows), and anti–bovine serum albumin binding (asterisk). Lanes 5 and 6 were incubated with patient B serum. They show 32 kd IE-specific binding and 68 kd binding (arrows). Lanes 7 and 8 were incubated with patient C serum. They show 32 kd IE-specific antigen and 68 kd and 50 kd binding (arrows).

(CHAPS), 4) 1% Triton X-100 (polyoxyethylene(9-10)*p-t*-octyl phenol), and 5) 0.5% sodium dodecyl sulfate (SDS). The extracts were then centrifuged at 1,000*g* for 10 minutes at 4°C, and the supernatants were saved for further investigation, while the pellets were discarded. After the protein content of each antigen preparation was determined by the bicinchoninic acid method (Pierce Biochemicals, Akron, Ohio), the concentration of all fractions was adjusted to 1.3 mg/mL by dilution in the aforementioned buffer, or through concentration using a Minicon B15 (Amicon, Beverly, Mass).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Western Blotting. The details of the methods are the same as described in a previous report.[2] In brief, SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli,[5] using SDS-containing 8% separating and 5% stacking slab gels. After all ex-

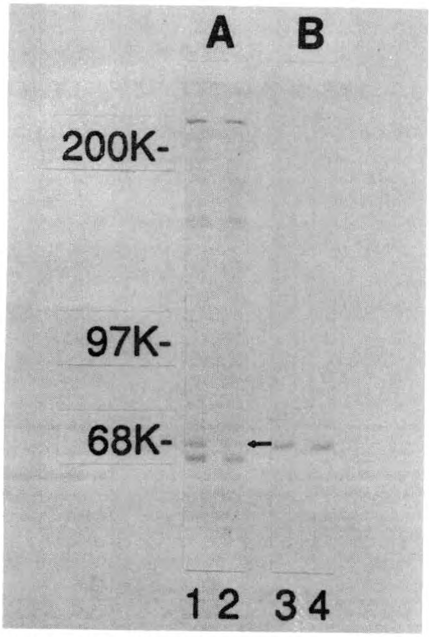

Fig 2. Absorption of anti–bovine serum albumin (BSA) antibody with BSA in excess. Crude inner ear extracts were used as antigen. Lane 1 is patient A serum, and lane 2 is patient A serum incubated with 1% BSA. Lane 3 is patient B serum, and lane 4 is patient B serum incubated with 1% BSA. Note absence of anti-BSA antibody reaction from lane 2 after absorption (arrow).

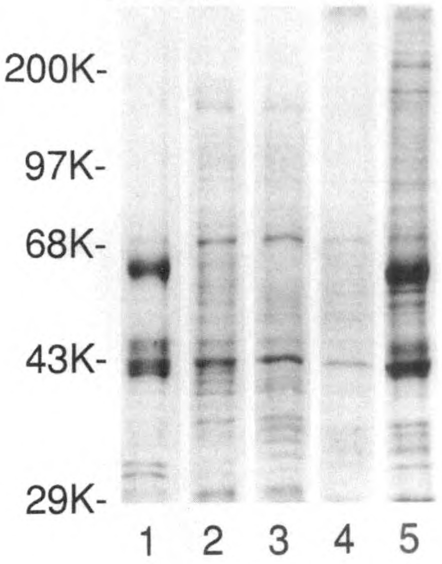

Fig 3. Coomassie blue staining protein patterns of all extracts from precipitates, which were solubilized by following solutions: 1% Triton X-100 (lane 1), 1% CHAPS (see text for definition; lane 2), 0.5 mol/L NaCl (lane 3), 0.15 mol/L NaCl (lane 4), and 0.5% SDS (lane 5).

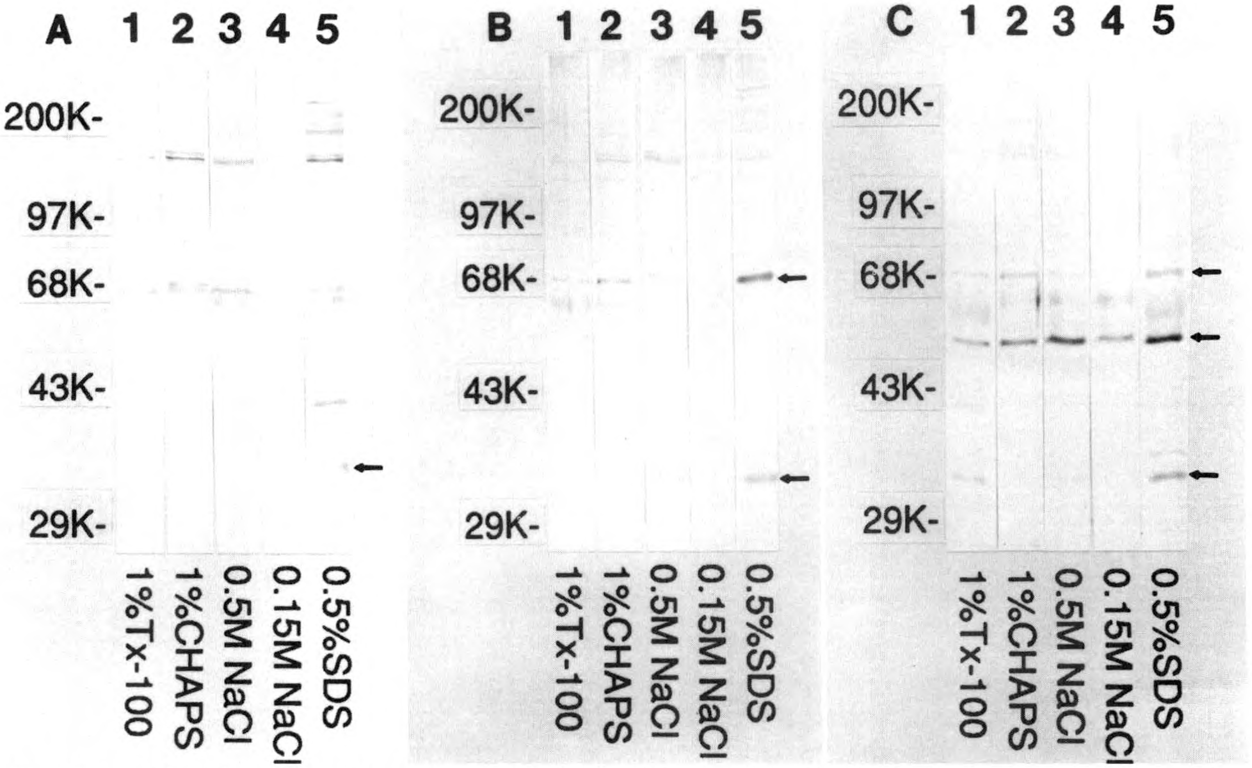

Fig 4. Immunoblots of sera from patients A through C (with idiopathic sensorineural hearing loss) against same extracts as in Fig 3. A) Serum from patient A, showing 33 to 35 kd inner ear (IE)–specific binding (arrow). B) Serum from patient B, showing 32 kd IE-specific binding and 68 kd binding (arrows). C) Serum from patient C, showing 32 kd IE-specific antigen and 68 kd and 50 kd binding (arrows).

tracts were dissolved in the sample buffer containing 3% 2-mercaptoethanol (approximately 1 mg/mL) and heated at 95°C for 5 minutes, 10 µg of protein was placed on each lane. The slab gels were electrophoresed at 200 V for approximately 40 minutes. Following electrophoresis, representative gels were stained with Coomassie brilliant blue R250, and the others were employed in Western blotting, according to the method of Towbin et al.[6] After the gels were subjected to SDS-PAGE, the separated proteins were electrophoretically transferred to nitrocellulose membrane at 100 V for 1 hour. The blots were incubated for 2 hours at room temperature in 5% dry milk and 0.2% Tween 20 in phosphate-buffered saline, pH 7.4, and overnight at 4°C in a 1:1,000 dilution of patient's serum that was diluted in 1% dry milk and 0.1% Tween 20 in phosphate-buffered saline. The blots were then rinsed and incubated for 2 hours at room temperature with a 1:5,000 dilution of peroxidase-labeled rabbit anti human immunoglobulin G gamma chain used as the secondary antibody in the above buffer. After appropriate washing, the bound peroxidase-labeled secondary antibody was reacted with the substrate 3,3′-diaminobenzidine by using 20 mg/33 mL of the 0.05 mol/L Tris, 0.2 mol/L NaCl, pH 7.4. Hydrogen peroxide 0.03% was used with the substrate, and the reaction was terminated by washing in distilled water.

RESULTS

Figure 1 shows the protein and immunoblot staining patterns of the water-soluble and SDS-soluble fractions produced following incubation with sera from representative patients A through C. The staining pattern of the SDS-soluble fraction was distinctly different from that of the water-soluble fraction. As reported previously,[2] the serum from patient A recognized 33 to 35 kd IE-specific antigen, 62 kd antigen, and bovine serum albumin (BSA); the serum from patient B recognized 68 kd antigen and 32 kd IE-specific antigen; and the serum from patient C recognized 68 kd and 50 kd antigens and 32 kd IE-specific antigen. Each of these antigens, except BSA, were insoluble in 50 mmol/L Tris buffer, but dissolved in 0.5% SDS, 50 mol/L Tris buffer.

In order to distinguish 68 kd IE antigen from BSA, which has a similar molecular weight but appears slightly lighter on the gel, we absorbed out anti-BSA antibody activity in patients' sera by incubation with 1% BSA in excess. After absorption, the serum from patient A that had anti-BSA antibody lost reactivity at 68 kd, while the serum from patient B that had anti–68 kd protein antibody still showed reactivity to 68 kd antigen (Fig 2).

Coomassie blue staining patterns of the fractions

TABLE 1. SUMMARY OF EXTRACTION TECHNIQUES FOR IMMUNIZATION IN EXPERIMENTAL AUTOIMMUNE LABYRINTHITIS

Authors	*Date*	*Animal Source*	*Inner Ear Source*	*Extracting Solution*	*Centrifuge Speed*	*Supernatant or Precipitate*
Terayama and Sasaki[11]	1964	Guinea pig	Whole	PBS	NP	Whole
Harada et al[12]	1984	Rabbit	Lateral wall	H_2O	3,000 rpm for 5 min	Precipitate
Harris[3]	1987	Bovine	Whole	PBS	2,500 rpm for 10 min	Supernatant
Soliman[13]	1989	Guinea pig	Whole	H_2O	3,000 rpm for 5 min	Precipitate
Orozco et al[14]	1990	Chick or guinea pig	Whole	HBSS	NP	Whole

PBS — phosphate-buffered saline, HBSS — Hanks' balanced salt solution, NP — no description provided.

extracted with the aforementioned solutions and detergents showed that there were several distinct protein patterns suggesting that the solubility of IE tissue proteins will vary with the extracting solutions adopted (Fig 3). Using the binding characteristics of sera from patients A through C in immunoblotting with antigen preparations derived from the different extraction procedures, we recovered the 68 kd antigen in highest concentration with the 1% CHAPS extraction and found it to be absent in the 0.15 mol/L NaCl extraction (Fig 4). The 62 kd and 50 kd antigens were recovered by all extracting solutions. In contrast, the 33 to 35 kd IE-specific antigen was extracted only in 0.5% SDS. The 32 kd IE-specific antigen was recovered in 0.5 mol/L NaCl, 1% Triton X-100, and 0.5% SDS, but not in 0.15 mol/L NaCl or 1% CHAPS.

DISCUSSION

Numerous reports have now emerged that employ preparations of IE proteins used as antigens for clinical and experimental investigation.[1,7-10] The techniques currently being employed for these purposes are summarized in Tables 1[3,11-14] and 2.[1,7-10] It should be evident that the multiple sources of antigen that have been used previously may result in a variability in antigenicity. Additionally, when one is attempting to establish an animal model of AIED, the result will greatly depend on several factors: how the antigen is prepared, how disparate the source of antigen is from the recipient, and the route and schedule of immunizations. For instance, isologous antigen is a poor immunogen compared to heterologous antigen, and obviously there will be a great difference in the protein prepared from a simple homogenation of the whole tissue versus the use of either the precipitated fractions following centrifugation or the supernatant.[3,11-14] While one empirically has reasons for selecting a particular method, it may be dangerous to assume that the antigens of interest have been captured without specifically analyzing and comparing the results of the various techniques employed. Therefore, further research in this area should carefully report the extraction techniques employed and their success in protein recovery. In addition, in studies of AIED the parameters of immunization should be well defined and standardized, and the antigen preparation should be highly refined so that reproducible and comparable results can be achieved. This is particularly important since diagnostic and therapeutic decisions are being formulated on the basis of the results of these tests.

The IE tissues in clinical use are usually harvested from human temporal bones, homogenized in PBS, and centrifuged at relatively high speed, and the supernatant is used as antigen for immunoassays. However, these procedures do not liberate many of the insoluble proteins, such as those that are membrane-associated, into aqueous solution; as a result, they may be discarded in the precipitate. In this study, we demonstrated that the solubility of 33 to 35 kd IE-specific antigen, 32 kd IE-specific antigen, and 68 kd antigen was limited, and that these antigens did not dissolve in 0.15 mol/L NaCl Tris buffer that had nearly the same ionic strength as physiologic saline. Thus, when physiologic saline is used as an extraction buffer, these IE antigens may not be solubilized. Therefore, in such a case, a misleading result might ensue if the target of the autoimmune disorder was indeed these antigenic epitopes.

Storage and handling of tissue may also have an

TABLE 2. SUMMARY OF EXTRACTION TECHNIQUES FOR IMMUNOASSAYS IN CLINICAL INVESTIGATION

Authors	*Date*	*Source of Antigens*	*Inner Ear Source*	*Extracting Solution*	*Centrifuge Speed*	*Supernatant or Precipitate*
McCabe[7]	1979	Human	Whole	NP	NP	NP
Hughes et al[8]	1984	Human	Whole	PBS	20,000*g*	Supernatant
Arnold et al[9]	1985	Human	Whole	NP	NP	NP
Veldman[10]	1988	Human	Whole	PBS	20,000*g*	Supernatant
Harris and Sharp[1]	1990	Bovine	Whole	PBS	2,500 rpm for 10 min	Supernatant

NP — no description provided, PBS — phosphate-buffered saline.

effect on the proteins recovered. For instance, freeze-thawing several times disrupts membranes, possibly leading to the release of membrane-associated proteins into the buffer. If the tissues are then washed, these proteins will be lost.

An additional problem may be the contamination of the antigen preparation with common tissue or serum proteins. This has been a particular problem for us, since the putative antigen of interest was found to have a molecular weight of 68,000 kd, as does BSA. Fortunately, we found that BSA could be removed from the antigen preparation by taking advantage of its water solubility. Therefore, as a first step to release some proteins, including BSA, into aqueous solution, we used 50 mmol/L Tris buffer and a hand homogenizer in order to osmotically and mechanically disrupt the cell membranes. This was then followed by centrifugation to remove insoluble materials. Sequential extractions were then needed, since the antigens of interest remained in the precipitate.

In general, to extract insoluble proteins, several methods have been developed, using solutions of high ionic strength or high or low pH, solutions with added ethylenediaminetetraacetic acid, organic solvents, enzyme digestion, detergents, or a combination of these.

In this study we employed solutions of different ionic strengths and detergents (CHAPS, Triton X-100, and SDS). CHAPS is an amphoteric detergent, Triton X-100 is a nonionic detergent, and SDS is an anionic detergent. Of these, SDS has the greatest ability to solubilize insoluble proteins; however, it also has a tendency to denature them and cause them to lose their antigenicity. In contrast, CHAPS has the least solubilization capability; however, it will not denature proteins. Triton X-100 lies somewhere between the two for these characteristics. The results of this study indicate that a 32 kd IE-specific antigen is solubilized in 1% Triton X-100 Tris buffer, 0.5% SDS Tris buffer, or 0.5 mol/L NaCl Tris buffer solutions, and that a 68 kd antigen is solubilized in 1% Triton X-100 Tris buffer, 1% CHAPS Tris buffer, 0.5% SDS Tris buffer, or 0.5 mol/L NaCl Tris buffer solutions, while a 33 to 35 kd IE-specific antigen is solubilized only in 0.5% SDS Tris buffer solution. We also found that the 33 to 35 kd IE-specific antigen is solubilized by a combination of 0.5% Triton X-100 and 0.5 mol/L NaCl Tris buffer (data not shown). While these methods represent commonly employed extraction protocols, there are other and perhaps better techniques to be used for these antigens.

Since Yoo et al[15] reported autoimmunity against type II collagen in otosclerosis and Meniere's disease in 1982, it has been thought that type II collagen is one of the most important and prevalent IE antigens. None of the antigens mentioned above, however, showed any identity with type II collagen standards employed simultaneously.

The exact nature of these IE proteins (antigens) of interest is as yet unknown, despite their clinical and diagnostic importance and their indispensability to research in the field of IE autoimmunity. They might be intracellular proteins, biologic membrane–associated proteins, or a component of the otic capsule of the cochlea. In order to elucidate this further, purification of reasonable quantities of antigen will be required in order that amino acid sequencing for exact identification can be carried out. Once identified, there are several means by which these antigen(s) can be synthesized, obviating the need for future harvesting of IE tissues. This in turn will facilitate any diagnostic procedure and open up new investigative avenues for the treatment of AIED.

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20TH INTERNATIONAL CONFERENCE ON COCHLEAR IMPLANTS

The 20th International Conference on Cochlear Implants will be held June 23-25, 1993, at the Ritz-Carlton Hotel, San Francisco, California. For further information, contact University of California, Extended Programs in Medical Education, Room LS-105, San Francisco, CA 94143-0742; (415) 476-4251.

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FOURTH INTERNATIONAL SYMPOSIUM AND WORKSHOPS ON INNER EAR MEDICINE AND SURGERY

The Fourth International Symposium and Workshops on Inner Ear Medicine and Surgery will be held July 17-24, 1994, in Snowmass-Aspen, Colorado. For more information, please contact I. Kaufman Arenberg, MD, Executive Director and Program Chairman, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; phone 303-788-4320 or 303-850-9545; fax 303-788-4234.

Ann Otol Rhinol Laryngol 102:1993

LARYNGEAL BRAIN STEM EVOKED RESPONSE IN THE PORCINE MODEL

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Exaggeration of normally protective laryngeal reflexes is thought to play a role in several disorders, including the sudden infant death syndrome. An analysis of brain stem neural activity following laryngeal stimulation may provide insight into the pathophysiology of pathologic laryngeal reflexes and help to identify individuals at risk for these disorders. The purpose of this study was to define the far-field brain stem activity following laryngeal stimulation in the porcine model. This activity has been termed the laryngeal brain stem evoked response and may represent a potentially useful and objective measure of the neuronal activity in the laryngeal reflex pathway. Electrical stimulation of the superior laryngeal nerve was performed in 14 mixed-breed piglets under a variety of physiologic conditions. A total of six positive and six negative discrete waves were detected, with mean latencies ranging from 1.24 to 7.16 milliseconds. Stimulations performed during hypoxic, hypercapneic, or hypocapneic conditions resulted in no significant differences in waveform latencies. There appears to be a reproducible, but somewhat variable, brain stem response elicited by superior laryngeal nerve stimulation that can be recorded via a far-field technique in the porcine model.

KEY WORDS — brain stem, chemoreflex, evoked potentials, larynx, sudden infant death syndrome, superior laryngeal nerve.

INTRODUCTION

Laryngeal reflexes play an important role in respiratory control and pulmonary protection from aspiration. Chemical or mechanical stimulation of the supraglottic mucosa or direct electrical stimulation of the superior laryngeal nerve (SLN) may result in two responses (Fig 1). The laryngeal adductor response causes glottic closure.[1,2] The second response, the laryngeal chemoreflex (LCR), results in centrally mediated apnea, hemodynamic changes, and swallowing.[3] There is evidence that these laryngeal reflexes, when hypersensitive, may be responsible for several disorders, including spasmodic dysphonia, idiopathic laryngospasm, "reflex" apnea, apnea of infancy, and sudden infant death syndrome (SIDS).[4-8]

Previous studies have attempted to identify the factors that regulate the intensity of laryngeal reflex activity. The magnitude of the LCR has been shown to be age-dependent and influenced by hypoxia.[8-13] In the piglet, maturational changes involving the brain stem nuclei controlling respiration are believed to be responsible for the age dependence of the LCR.[14] Anatomic studies of the SLN have demonstrated postnatal maturational variations in its projections to respiratory center nuclei that parallel the changes in the apneic and cardiovascular responses of the LCR.[9] These brain stem nuclei may play an integral role in mediating pathologic laryngeal reflexes. Thus, an objective measurement of the brain stem activity following SLN stimulation may provide valuable insights into the pathophysiology of laryngeal reflex dysfunction.

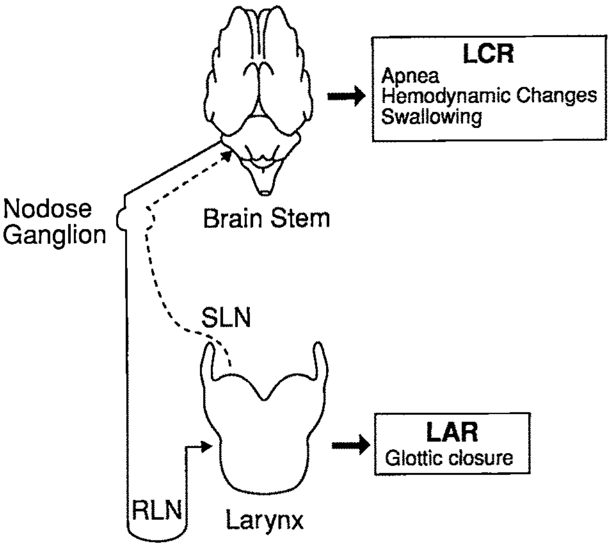

Fig 1. Diagrammatic representation of laryngeal reflexes resulting from supraglottic or direct superior laryngeal nerve (SLN) stimulation. RLN — recurrent laryngeal nerve, LCR — laryngeal chemoreflex, LAR — laryngeal adductor response.

From the Department of Otolaryngology–Head and Neck Surgery, University of Michigan, Ann Arbor, Michigan. Supported by the Percy Memorial Research Grant of the American Academy of Otolaryngology–Head and Neck Surgery Foundation, Inc. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan.

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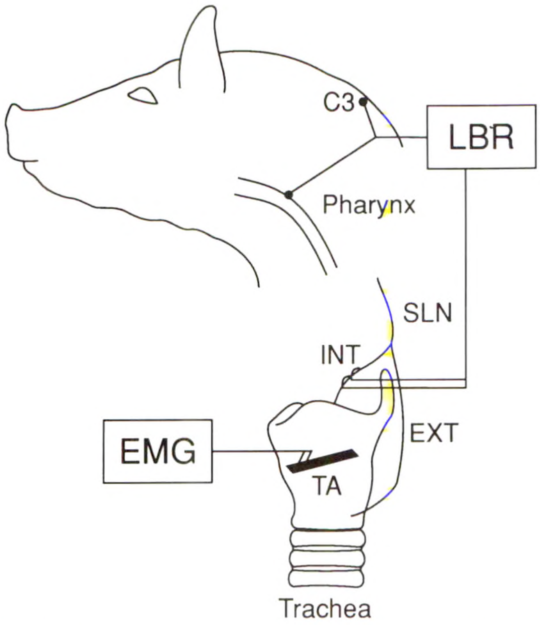

Fig 2. General animal preparation. Laryngeal brain stem evoked response (LBR) was recorded from pharynx-cervical (C3) montage after stimulation of superior laryngeal nerve (SLN). Thyroarytenoid (TA) muscle electromyographic response (EMG) was also obtained. INT — internal, EXT — external.

Brain stem nuclei involved in laryngeal reflex activity have been identified by means of invasive recordings from the brain stem following direct electrical SLN stimulation. These nuclei include the solitary tract nucleus, the reticular formation (including respiratory center nuclei), and the nucleus ambiguus. Although there have been several near-field reports detailing this polysynaptic reflex pathway, there are only a few preliminary studies that have attempted to obtain far-field data with relatively noninvasive techniques. In 1987 Isogai et al,[15] using a far-field technique in adult cats, described a method to elicit averaged electrical activity evoked by SLN stimulation from the brain stem. They designated these recordings the laryngeal brain stem evoked response (LBR). Signal averaging is used to improve the signal to noise ratio, as with sensory evoked potentials. Synchronized neuroelectrical activity of the nuclei evoked by the laryngeal reflex may result in a series of far-field potentials recorded by means of surface electrodes. Isogai et al recorded four positive and four negative potential peaks in the feline LBR, with latencies ranging from 0.5 to 5.5 milliseconds. The LBR was further described in adult and infant cats by Anonsen et al[16] and Lalakea et al.[17]

Despite the prior reports, there are several unanswered questions that serve as the primary focus of the present study. The purpose of this study is to 1) verify that the LBR is a neurologic response generated by brain stem nuclei mediating laryngeal reflexes, 2) demonstrate that the response can be recorded outside the feline model, 3) determine the most consistent and robust LBR components, 4) evaluate the effect of varying physiologic parameters on LBR peak latencies, and 5) determine the degree of contamination by myogenic artifact on the LBR. Maturational changes in the brain stem nuclei responsible for differential LCR responses should be manifested as variations in LBR latencies. If the LBR turns out to be a reliable indicator of laryngeal reflex sensitivity, it may become a mechanism for identifying and evaluating subjects predisposed to pathologic laryngeal reflexes.

METHODS

Experimental Setup. Fourteen mixed-breed piglets, 30 to 45 days of age and 7 to 12 kg in weight, were chosen for this study because fatal laryngeal reflex apnea does not occur in piglets more than 21 days old.[8] All piglets were premedicated with intramuscular Telazol (6 mg/kg), Rompun (3 mg/kg), and atropine sulfate (0.04 mg/kg). Anesthesia was maintained with intramuscular ketamine hydrochloride (10 mg/kg) administered every 30 minutes. A heating pad, femoral arterial and venous catheters, and a rectal thermometer were placed. Periodically, blood was taken from the arterial line to measure the P_{O2}, P_{CO2}, and oxygen saturation. The larynx and trachea were exposed through a vertical midline cervical incision. The trachea was entered below the second ring. A cuffed endotracheal tube was inserted into the lower trachea to provide ventilation during muscular paralysis. The blood pressure and heart rate were recorded with a Hewlett Packard model 7754B chart recorder.

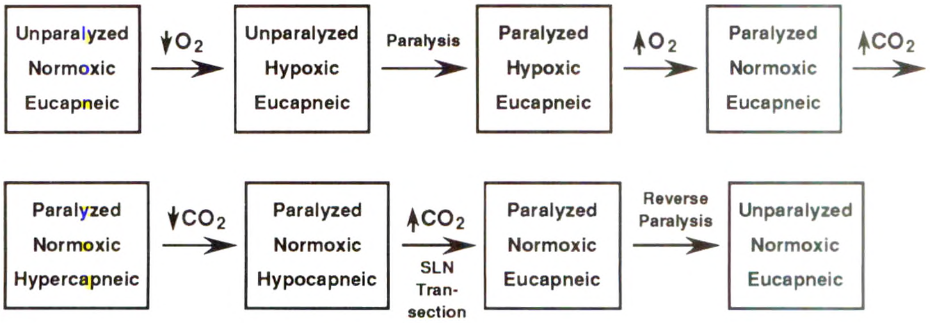

Fig 3. Experimental design. Laryngeal brain stem evoked responses were recorded during physiologic conditions represented by each box in sequence shown. SLN — superior laryngeal nerve.

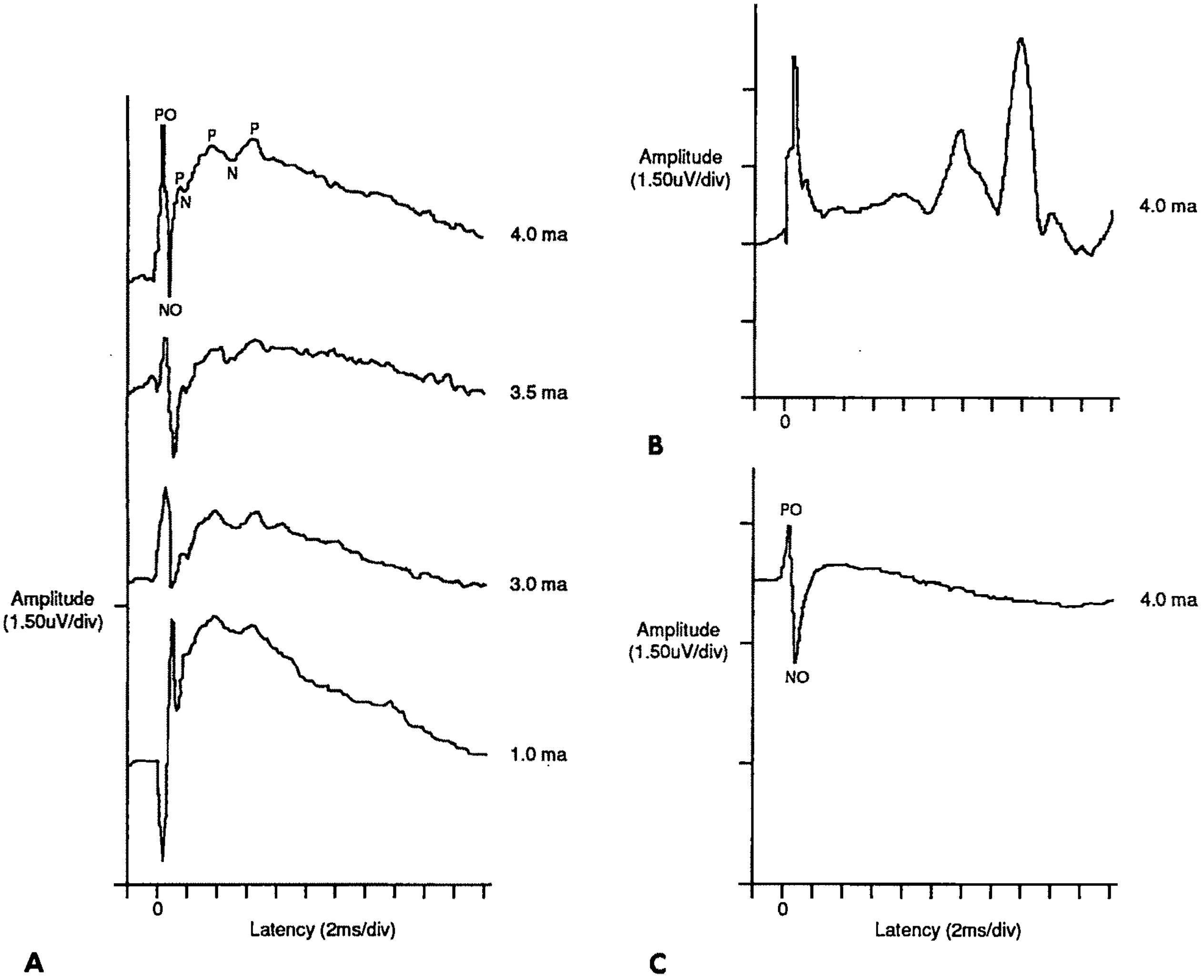

Fig 4. Laryngeal brain stem evoked response waveforms. These tracings were recorded from same piglet under following physiologic conditions. A) Paralyzed, normoxic, eucapneic state. B) Unparalyzed, normoxic, eucapneic state. C) Paralyzed, normoxic, eucapneic state after transection of superior laryngeal nerve. Stimulus intensities are listed in milliamperes (ma). P — positive peak, N — negative peak. P0, N0 — stimulus artifact.

The left SLN was isolated, and the thyrohyoid membrane was divided. Grass type E2 platinum subdermal needle electrodes were used to record the brain stem activity and the laryngeal electromyographic (EMG) response (Fig 2). The noninverting (active) electrode (P) was placed into the posterior pharyngeal wall. The reference electrode (C3) was secured in the subcutaneous tissue of the posterior neck at the level of the third cervical vertebra. This electrode pair formed a pharynx-C3 (P-C3) recording montage flanking the caudal brain stem. This montage has been found to be effective for recording brain stem activity.[16] Two needle electrodes were inserted into the left thyroarytenoid muscle for EMG recordings. A ground electrode was placed in the left lower extremity. Two Teflon-insulated platinum-iridium wires (Medwire-3T) were hooked around the internal branch of the left SLN to provide bipolar stimulation to the afferent limb of the reflex arc.

Laryngeal Brain Stem Evoked Response Measurement. A Bio-Logic Navigator electrodiagnostic testing system (Bio-Logic Systems Corp) was used to deliver the electrical stimulus to the SLN and to record both the brain stem response from the P-C3 electrode montage (channel 1) and the thyroarytenoid EMG response (channel 2) after stimulation. In the unparalyzed state, evoked compound motor potential recordings from the thyroarytenoid muscle were used to demonstrate an intact laryngeal reflex arc in each piglet. This EMG response served as a positive control by documenting the adequacy of the SLN stimulus to activate laryngeal reflex efferents. The absence of this response was used to verify the efficacy of neuromuscular blockade.

Monopolar rectangular constant current pulses (0.1 millisecond) of alternating polarity were delivered at a rate of 11/s for stimulation purposes. The current level was varied from 0 to 12 mA, and each averaged LBR response was derived from 1,500 averaged

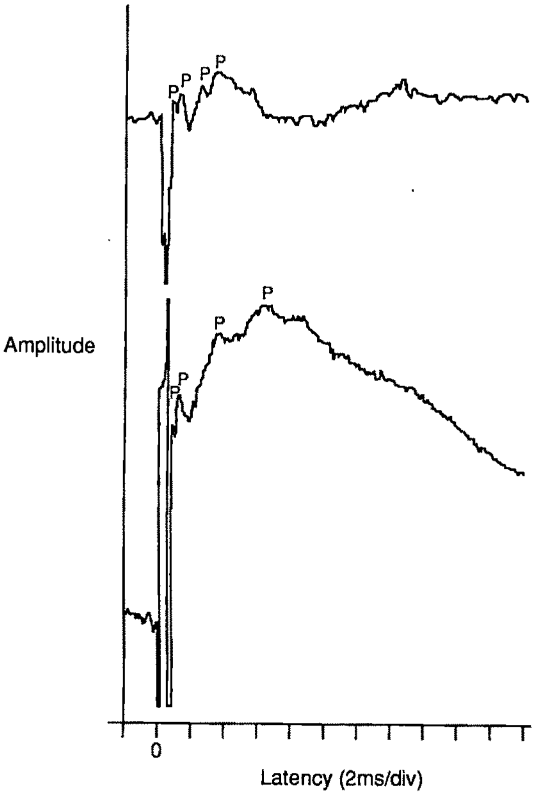

Fig 5. Waveform variability of laryngeal brain stem evoked response. Shows two responses obtained under similar stimulus conditions in different piglets, demonstrating inconsistencies in morphology of peaks. P — positive peak.

sweeps. The activity recorded by the P-C3 montage was amplified 100,000 times. The compound motor action potentials from the thyroarytenoid muscle were amplified 500 times. Both responses were filtered with a band pass of 10 to 1,500 Hz. A relatively low high-pass filter setting was selected to avoid the ringing artifact derived from the electrical stimulus. In several piglets, an auditory brain stem evoked response to click stimuli delivered to the left ear was also recorded from the P-C3 montage. This provided a control measure by demonstrating that the recording montage could effectively detect brain stem activity, and it verified the physiologic integrity of the brain stem.

The sequence of physiologic states during which stimulations were performed is illustrated in Fig 3. Initial recordings were obtained while each piglet was spontaneously breathing under normoxic (oxygen saturation greater than 90%), eucapneic (Pco2 between 36 and 44 mm Hg) conditions. The fractional inspiratory concentration of oxygen was then lowered by admixing nitrous oxide, and stimulations were repeated under hypoxic (oxygen saturation less than 70%) conditions. The piglets were then completely paralyzed with intravenous pancuronium bromide (0.3 mg/kg) and mechanically ventilated with a Harvard respiration pump (model 607). In both the normoxic and hypoxic states several recordings were obtained under eucapneic (Pco2 between 36 and 44 mm Hg), hypercapneic (Pco2 greater than 55 mm Hg), and hypocapneic (Pco2 less than 25 mm Hg) conditions at various stimulus intensity levels. After LBR data collection, the SLN was transected proximal to the stimulating electrodes, and the stimulation was repeated. This provided a negative control and served to isolate the stimulus artifact.

The LBR consisted of positive and negative peaks following the stimulus artifact. Only those peaks that were unanimously identified and marked by three of the authors, who independently analyzed the tracings, were considered. A series of sequential latency intervals were defined. In order to be consistent with peak numbering and allow for meaningful intersubject comparisons, waves were labeled according to the latency interval in which they occurred. For example, positive peaks with a latency of 2.7 to 4.2 milliseconds were designated as P3. To determine the most consistent LBR peak latencies, the occurrence rate of each peak and the coefficient of variation for its latency were calculated. Simple linear regression analysis was used to evaluate the effect of varying the stimulus intensity on LBR peak latencies. Analysis of variance techniques were used to determine the impact of altering the physiologic state on LBR peak latencies. Homoscedasticity was assured by Bartlett's test for homogeneity of group variances. Tukey's procedure was used to control for the effects of multiple comparisons.

RESULTS

Twelve of 14 piglets demonstrated clear and robust LBR tracings. Absolute peak latencies were the primary focus of attention. Wave amplitudes were too highly variable, even under identical stimulus conditions in the same piglet, for reliable comparisons. The effect of varying the stimulus intensity on the LBR peaks was initially evaluated. Since changes in the suprathreshold stimulus intensity produced negligible variation in the peak latencies, all LBR trials were grouped according to physiologic condition for further evaluation. A total of 100 LBR tracings were recorded in various physiologic states.

Figure 4A shows characteristic LBR waveforms in the paralyzed, normoxic, eucapneic state, which represents the "control" state. Also illustrated, in Fig 4C, is the effect of sectioning the SLN proximal to the stimulating electrodes. The LBR waveforms consisted of a series of positive and negative peaks representing laryngeal reflex neuronal activity. Only the initial peaks (P0, N0) corresponding to the stimu-

TABLE 1. LARYNGEAL BRAIN STEM EVOKED RESPONSE PEAKS

Wave	*Latency Range (ms)*	*Mean Latency (ms)*	*SD*	*% Occurrence*	*Coefficient of Variation*	*N*
P1	0.93-1.61	1.24	0.19	71	0.16	44
N1	1.52-2.10	1.82	0.13	85	0.07	53
P2	2.20-2.69	2.52	0.16	15	0.06	9
N2	2.98-3.18	3.05	0.10	11	0.03	7
P3	2.73-4.09	3.37	0.36	65	0.11	40
N3	3.98-5.23	4.71	0.38	18	0.08	11
P4	4.45-5.60	4.97	0.54	6	0.11	4
N4	5.13-6.20	5.47	0.37	13	0.07	8
P5	5.90-6.50	6.25	0.23	27	0.04	17
N5	6.09-7.38	6.86	0.29	27	0.04	17
P6	6.69-8.06	7.16	0.49	15	0.07	9
N6	7.67-8.16	7.89	0.22	6	0.03	4

lus artifact remained after SLN transection. A total of six positive (P1 through P6) and six negative (N1 through N6) peaks were detected in LBR waveforms obtained in the control state. However, there was some inconsistency in the configuration of some of these peaks, and no single tracing contained all these peaks (Fig 5). The intersubject reproducibility of LBR waveforms in the control state is represented in Table 1, which lists the frequency of peak occurrence and the coefficient of variation for each peak latency. The coefficient of variation for all component peak latencies was less that 0.2; however, only P1, N1, and P3 were present in greater than 50% of the tracings.

Absolute peak latencies obtained in the control state were compared to those recorded when the arterial oxygen and carbon dioxide contents were varied. Stimulation under either hypercapneic or hypocapneic conditions produced no significant changes in any of the peak latencies. Hypoxia produced a statistically significant difference in only P1 latency. Mean P1 latency in the control state was 1.24 milliseconds, while that obtained during hypoxic conditions was 1.04 milliseconds ($p = .001$). The effect of muscular artifact on the LBR tracing is demonstrated in Fig 4. Figure 4B is an LBR waveform obtained in the unparalyzed state, while Fig 4A represents tracings recorded from the same piglet during complete muscular paralysis. Muscular contamination caused some distortion of the earlier LBR waves and produced additional late peaks in the 12 to 20 millisecond range, which coincides with the latency of the compound motor action potential.

DISCUSSION

Laryngeal reflexes provide some control over respiration and protection from aspiration. These functions are vital to the survival of the organism, but under certain circumstances these reflexes can become pathologic and lead to several disorders. A hyperactive laryngeal adductor response can cause spasmodic dysphonia. The LCR, which results in centrally mediated apnea, hemodynamic changes, and swallowing following laryngeal stimulation, may become pathologic in susceptible subjects, resulting in prolonged or fatal apnea.[3,8] There is evidence that an exaggerated LCR response may underlie several disorders, including "reflex" apnea, apnea of infancy, and SIDS.[4-8] A measure of laryngeal reflex sensitivity would be of benefit in evaluating subjects with pathologic reflexes. An analysis of the brain stem neural activity resulting from laryngeal stimulation may provide an indicator of laryngeal reflex sensitivity.

The brain stem activity resulting from direct electrical stimulation of the SLN has been recorded by means of a far-field technique in preliminary reports using a feline model.[15-17] Isogai et al[15] termed this activity the LBR. The present study attempted to characterize the LBR under more strictly controlled conditions than used previously. In addition, by obtaining an LBR in the porcine model, the existence of the response across species is demonstrated. Signal averaging was used to obtain the far-field LBR evoked by SLN stimulation. The same electrode montage and similar stimulus parameters that had been found useful for recording laryngeal evoked potentials in prior reports was used. The fact that a typical auditory brain stem response was successfully recorded from the P-C3 montage used in this study demonstrates its effectiveness in detecting brain stem activity.

The waveforms obtained in this study are believed to represent LBR neural activity initiated by SLN stimulation for the following reasons. First, the initial peak occurred after SLN stimulation in each observation. Consistent responses to stimuli of alternating polarity suggest that these waves represent nonrandom activity triggered by SLN stimulation. The latencies of the peaks preceded the thyroarytenoid compound motor action potential, thereby placing them in the appropriate time epoch. Transection of

TABLE 2. LATENCY COMPARISONS

Generator Source	*Near-Field Data (ms)*	*Present Study (ms)*	*Isogai et al*[15] *(ms)*	*Anonsen et al*[16] *(ms)*
Ipsilateral vagus nerve	1.1 ± 0.9[18]	P1 1.24 N1 1.82	P1 0.5-1.0 N1 1.2-1.4	P0 0.78 N0 1.10 P1 1.17
Solitary tract nucleus	2-4[19-21]	P2 2.52 N2 3.05 P3 3.37	P2 1.6-2.3 N2 2.4-2.7 P3 2.8-3.2	P2 2.37 N2 2.76 P3 3.39
Nucleus ambiguus	5-8[20,22]	N3 4.71 P4 4.97 N4 5.47 P5 6.25	P4 variable N4 5.0-5.5	P4 4.96 N4 5.52
Reticular formation	7[19]	N5 6.86 P6 7.16 N6 7.89		P5 6.65

the SLN abolished all the peaks except those consistent with the stimulus artifact. Finally, that the waveforms persisted despite complete muscular paralysis suggests a neurogenic origin for these far-field potentials.

The LBR tracings consisted of several positive and negative peaks within a 0.9 to 8.2 millisecond latency range. A comparison of the far-field data in this study with prior far- and near-field reports following electrical stimulation of the SLN in cats is shown in Table 2.[15,16,18-22] Although there may be some interspecies differences, speculation as to the generator sources of the LBR peaks can be made. All LBR peaks fell within the expected latency range for neural activity following SLN stimulation as revealed in the near-field recordings. P1 and N1, with mean latencies of 1.2 and 1.8 milliseconds, respectively, correspond well to the orthodromic conduction time from the SLN to the nodose ganglion. The latency of peaks P2, N2, and P3 may coincide with activity in the solitary tract nucleus. The conduction latency between the SLN and the nucleus ambiguus according to near-field data is similar to the latencies associated with the LBR peaks P4, N4, and P5. Last, P6 and N6 latencies appear to correspond to the activation time of the reticular formation. Nevertheless, the relationships between the LBR peaks and generator sources are merely suggestive, since firm conclusions regarding the anatomic origins of the LBR peaks would require lesion studies or stereotactic recordings from generators along the reflex pathway.

Although every effort was made to conform to sound electrophysiologic principles, there was considerable variation in interpiglet peak reproducibility. The most frequently occurring LBR peaks in the paralyzed, normoxic, eucapneic state were P1, N1, and P3. Several other peaks were present in a smaller fraction of tracings. The fact that the less frequently detected waves occurred at such characteristic and reproducible latencies suggests that they, like the more frequently occurring peaks, represent actual neural activity associated with SLN stimulation, as opposed to artifact. The variability in peak occurrences may be explained in different ways. Some variability may be due to the sensitivity of the recording montage for the individual waves. Placement of the recording electrodes in a different orientation or in closer proximity to the brain stem may eliminate the problem of detecting the less frequently observed neural signals, and needs further investigation. Another possible explanation for the variability relates to the complexity of the reflex itself. Studies measuring other responses modulated by the reticular formation, such as the vestibulo-ocular reflex, have demonstrated that the state of arousal has a significant impact on these reflexes.[23,24] Since the reticular formation plays a role in laryngeal reflexes, factors that influence reticular formation activity, such as the state of arousal and the level of anesthesia, could influence reflex function. This effect may be reflected in the LBR as variations in either the presence or latency of individual peaks, or both.

Physiologic conditions are believed to affect the sensitivity of laryngeal reflexes. The LCR has been shown to be influenced by hypoxia in previous studies involving various animal models.[8,13] In most cases, there is an exaggeration of LCR severity when stimulations are performed under hypoxic conditions, resulting in prolonged apnea and hemodynamic instability. In this study, the effect of variable physiologic states on the LBR was analyzed. The intersubject LBR peak latencies, with the exception of P1, remained stable despite variations in the physiologic conditions. This suggests that the LBR is unresponsive to changes in arterial oxygen and carbon dioxide concentrations. Thus, the effect of hypoxia on LCR severity may be attributable to a mechanism other than one that has a direct influence on laryngeal reflex sensitivity.

The importance of complete muscular paralysis when recording the LBR was observed in this study. Muscle artifact was shown to have a significant effect on the LBR tracings, resulting in extraneous peaks not observed with total paralysis. In the present study, complete muscular paralysis was ensured by demonstrating the loss of the thyroarytenoid muscle compound action potential response. In previous LBR reports, although paralytic agents were administered, there was no specific mechanism used to guarantee that complete muscular paralysis was achieved.[15,16] This fact may account for some of the differences between the LBR tracings in this study and those in prior reports. Several other factors that may also have contributed to differences include the animal model, the method of peak identification and labeling, and recording parameters such as filter settings, electrode placement, and temperature control.

The LBR may have significant potential in both research and clinical applications. Now that the porcine LBR is defined, an attempt should be made to characterize the LBR response in humans. The direction of subsequent studies should be to compare the LBR present in subjects susceptible to a prolonged LCR response with the LBR associated with subjects that do not exhibit an exaggerated response. If a correlation between LBR peak latencies and LCR sensitivity could be demonstrated, the LBR may prove useful in identifying and evaluating individuals susceptible to pathologic laryngeal reflex function.

CONCLUSION

A brain stem response representing neural activity following direct electrical stimulation of the SLN can be recorded by means of a far-field technique in the porcine model. This response consists of various peaks with highly consistent latencies. A comparison of the tracings in this study to prior near-field data suggests that the peak latencies correspond to activity in established nuclei involved in laryngeal reflexes. This far-field brain stem response may prove to be clinically useful for studying subjects with disorders related to laryngeal reflex dysfunction.

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EVALUATION OF BRAIN COLLATERAL CIRCULATION BY THE TRANSCRANIAL COLOR DOPPLER–GUIDED MATAS' TEST

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For the determination of the indication for reconstruction of the carotid artery in patients with head and neck cancer, a color Doppler–guided Matas' test was performed to examine brain collateral circulation and blood flow of the middle cerebral artery (MCA) and internal carotid artery (ICA). Both SSA-270A convex (3.75 MHz) and linear (3.75 MHz and 2.5 MHz) array probes (Toshiba Co, Ltd) were used for this investigation. A temporary occlusion test (60 seconds) by digital pressure on the common carotid artery was performed on 30 patients with head and neck cancer. The results were classified into four groups according to the relative flow volume (percent blood flow volume before the test) of the MCA: group A, with a flow volume of 87% or more; group B, with a flow volume between 72% and 86%; group C, with a flow volume between 57% and 71%; and group D, with a flow volume of 56% or less. The relative flow volume of the MCA was correlated with the ICA stump pressure and single photon emission computed tomography during a balloon Matas' test (ICA occlusion). This test seems to be useful in predicting the risk of carotid resection without reconstruction and in determining the indication for reconstruction of the carotid artery.

KEY WORDS — brain collateral circulation, carotid artery reconstruction, head and neck cancer, Matas' test, transcranial color Doppler.

INTRODUCTION

Treatment of the metastatic neck lymph nodes invading the common or internal carotid artery (ICA) is of great concern to head and neck cancer surgeons. Radical surgery including resection or reconstruction of the carotid artery may be able to control the local lesion, and improve the survival rate in patients without distant metastasis. It would be necessary, though, to evaluate the brain's collateral circulation in the circle of Willis to decide whether reconstruction of the carotid artery is indicated. For this purpose, the color Doppler–guided Matas' test (CDMT) has been performed to evaluate blood flow volume in the middle cerebral artery (MCA) and the ICA.

Examination of neurologic signs, brain angiography, single photon emission computed tomography (SPECT),[1-3] electroencephalography (EEG), ICA stump pressure,[4] ocular plethysmography[5] during Matas' test,[6] and the balloon Matas' test (BMT)[7,8] have been performed to evaluate brain collateral circulation previously, but real-time quantitative methods have not been reported. This paper describes the CDMT and the indications for reconstruction of the carotid artery.

SUBJECTS AND METHODS

We examined 30 patients (24 men and 6 women) to assess their suitability for undergoing radical neck dissection. Their mean age was 53 years (range 24 to 82). Both SSA-270A convex (3.75 MHz) and linear (3.75 MHz and 2.5 MHz) array probes (Toshiba Co, Ltd) were used for this investigation. A sample volume of pulsed wave Doppler was placed in a blood vessel of interest, the angle marker was set to the direction of the blood flow to correct an angle factor, and then the Doppler shift was measured. The blood flow velocity waveform was calculated from the Doppler shift by a microcomputer and visualized on a display. The maximum flow velocity of the waveform was traced by a digitizer, and the mean blood flow velocity was calculated. The cross-sectional area of the vessel was measured on a color image. Then the blood flow volume was calculated from the mean blood flow velocity and the cross-sectional area of the vessel.

Relative Blood Flow Volume in MCA During 60-Second Matas' Test. A color Doppler image and the waveform of blood flow velocity in the MCA were obtained simultaneously by using the linear (2.5 MHz) array probe in a region above the zygomatic arch, from 1 to 5 cm in front of the ear. The common carotid artery (CCA) was occluded completely by digital pressure for 60 seconds. The data were recorded on videotape. The integral value of the blood

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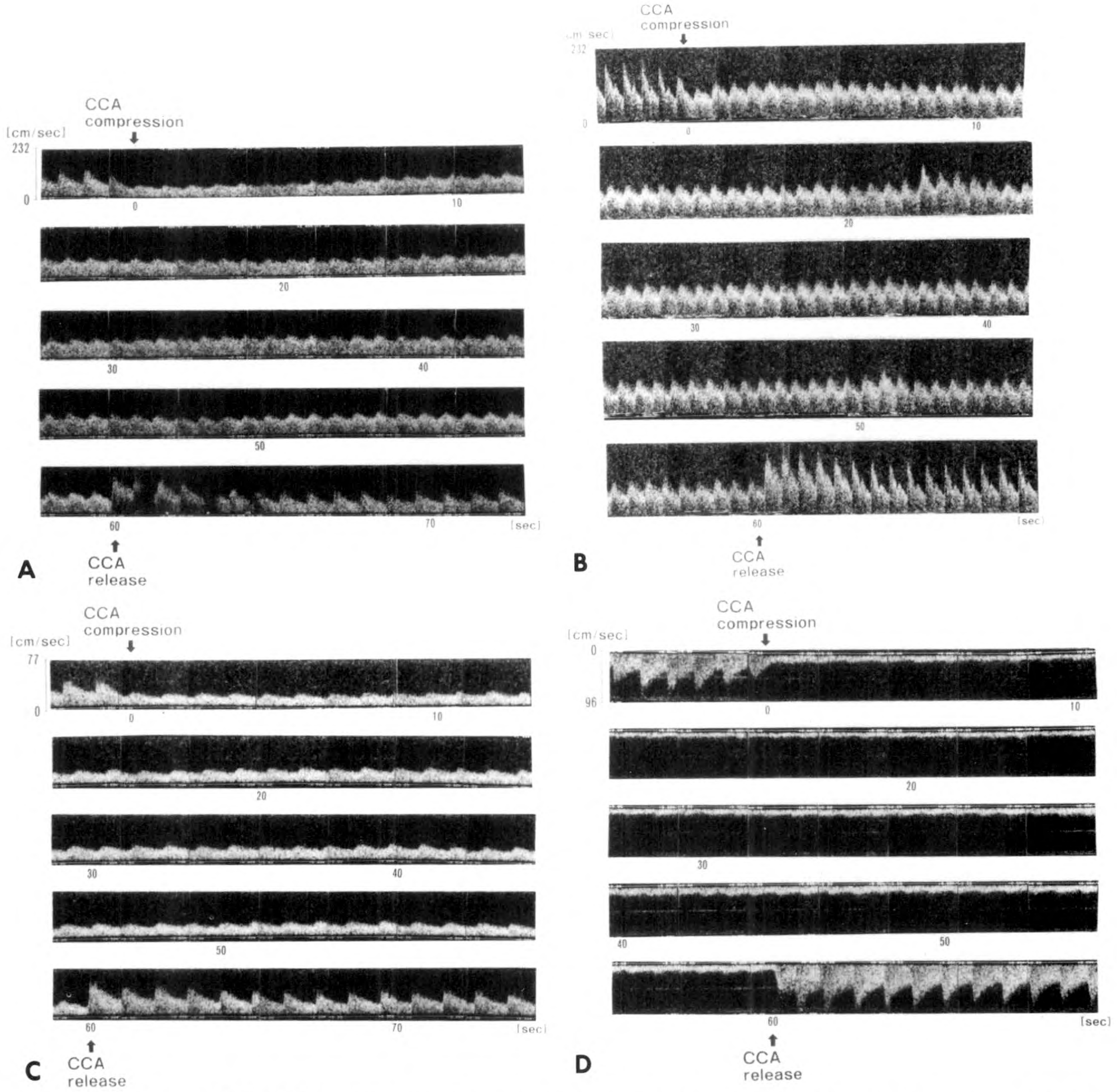

Fig 1. Blood flow velocity of the middle cerebral artery during 60-second Matas' test. A) Group A. Velocity was decreased temporarily after compression of common carotid artery (CCA) and gradually increased and pulsated in three cases. B) Group B. Velocity was fairly well restored in 12 cases. C) Group C. Velocity was poorly restored in 12 cases. D) Group D. Velocity remained reduced in three cases.

flow velocity over a 2.2-second period (length of the time axis of a single display) was calculated before and during the test by a digitizer (KD3800 Graphtec Co, Ltd). We defined the relative blood flow volume of the MCA before the test as 100%, and calculated the percent blood flow volume of the MCA during the 60-second Matas' test.

Increase of Blood Flow Volume in Contralateral CCA, ICA, and Bilateral Vertebral Arteries. We measured the increase in blood flow volume in the contralateral CCA, ICA, and bilateral vertebral arteries (VAs), which supplied blood to the brain during Matas' test.

Hemodynamics at Carotid Bifurcation During Matas' Test. Collateral circulation from the external carotid artery (ECA) to the ICA can occur during this test because of compression of the CCA. The ICA flow direction and volume were measured in 18 cases.

Richter's Anastomosis. The occipital artery, a branch of the ECA, is anastomosed to the VA directly or via a muscle branch for deep cervical muscles. This is called Richter's anastomosis.[9] It is an impor-

TABLE 1. INCREASE OF BLOOD FLOW VOLUME IN CONTRALATERAL CCA AND ICA AND BILATERAL VAs DURING MATAS' TEST

	*Increase (%)**	*n*	*Age**	*Male*	*Female*
Ipsilateral VA	50.4 ± 17.5	15	54 ±13	14	1
Contralateral VA	42.8 ± 18.9	6		5	1
CCA	40.6 ± 27.6	6	54 ± 9		
ICA	29.7 ± 7.9	6			

CCA — common carotid artery, ICA — internal carotid artery, VA — vertebral artery.

*Data are mean ± SD.

tant collateral pathway in occlusive carotid artery diseases. The hemodynamics of the carotid bifurcation in the case of a unilateral CCA ligation were investigated.

Comparison of CDMT and Balloon Matas' Test. A BMT was performed on seven patients with head and neck cancer. The selective ICA balloon occlusion test was performed after catheterization of the femoral artery using the Seldinger technique. A 15-minute occlusion test by balloon inflation was performed on the patients. The cross flow of the anterior communicating artery by brain angiography, stump pressure in the ICA, brain SPECT, EEG, and neurologic signs were evaluated during the BMT.

RESULTS

Relative Blood Flow Volume in MCA During 60-Second Matas' Test. The 30 patients were classified into four groups according to the relative flow volume in the MCA during the 60-second Matas' test. The relative mean flow was 72%, with an SD of 15%, meaning a mean plus 1 SD of 87% and a mean minus 1 SD of 57%. According to these, the patients were classified into group A, with a flow volume of 87% or more (three cases); group B, with a flow volume between 72% and 86% (12 cases); group C, with a flow volume between 57% and 71% (12 cases); and group D, with a flow volume of 56% or less (three cases). We present the velocity change in each group during the test (Fig 1). Only one patient, in group D, suffered any neurologic symptom, that being transient numbness of the contralateral hand. No other serious complications were encountered.

Increase of Blood Flow Volume in Contralateral CCA, ICA, and Bilateral VAs. The increase was 40.6% ± 27.6% in the contralateral CCA, 29.7% ± 7.9% in the contralateral ICA, 42.8% ± 18.9% in the contralateral VA (mean ± SD, n = 6), and 50.4% ± 17.5% in the ipsilateral VA (mean ± SD, n = 15; Table 1).

Hemodynamics at Carotid Bifurcation During Matas' Test. The results of this study are shown in Table 2. The direction of blood flow in the ICA was forward, from the ECA to the ICA, in 1 case, and reverse, from the ICA to the ECA, in 17 cases, but in 2 of the latter the direction changed to forward in a few minutes. The blood flow volume in the ICA ranged from 12 to 130 mL/min. There was no significant correlation between the reverse blood flow volume of the ICA and the relative flow volume of the MCA.

TABLE 2. HEMODYNAMICS AT BIFURCATION OF CAROTID ARTERY DURING MATAS' TEST

Case	*Age*	*Sex*	*MCA Flow (%)*	*Group**	*ICA Flow Volume (mL/min)*	*Flow Direction*
1	48	M	79	B	40	Forward
2	52	M	63	C	110	Reverse
3	46	M	78	B	80	Reverse, then forward
4	57	M	85	B	130	Reverse
5	67	M	83	B	102	Reverse
6	51	M	93	A	38	Reverse
7	62	M	63	C	48	Reverse, then forward
8	48	M	97	A	86	Reverse
9	26	F	89	A	65	Reverse
10	59	F	86	B	21	Reverse
11	59	M	82	B	64	Reverse
12	55	M	77	B	34	Reverse
13	36	M	75	B	130	Reverse
14	40	M	72	B	50	Reverse
15	73	M	70	C	130	Reverse
16	57	M	67	C	108	Reverse
17	55	M	61	C	12	Reverse
18	40	M	50	D	16	Reverse

MCA — middle cerebral artery, ICA — internal carotid artery.

*Defined in text.

Richter's Anastomosis. The direction of flow was reverse in the occipital artery, via Richter's anastomosis, in the unilateral CCA ligation. First, the hemodynamics at the carotid bifurcation were investigated. The flow direction in the ICA was reverse. The velocity and volume of blood flow in the ICA were 10 cm/s and 60 mL/min, respectively. The flow direction, velocity, and volume of blood flow in the ECA at the peripheral site of the occipital artery was forward, 39 cm/s, and 100 mL/min, respectively. Second, the flow direction was reverse in the occipital artery (Fig 2). The flow velocity and volume in the ECA at the central site of the occipital artery were 35 cm/s and 50 mL/min, respectively. In this case one half of the flow volume of the ECA was supplied from the VA via Richter's anastomosis.

Comparison of CDMT and BMT. The cross flow was good, the EEG was normal, and neurologic signs

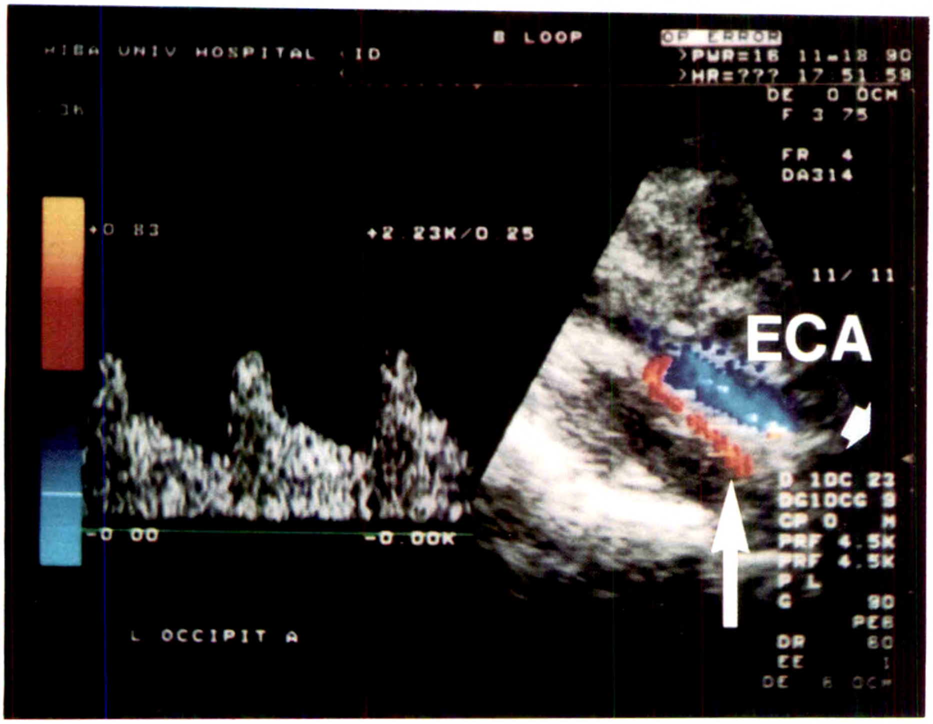

Fig 2. Reverse flow in occipital artery. Flow direction in external carotid artery (ECA, blue) was forward. Flow direction was reverse in occipital artery (long arrow, red). In this case, one half of flow volume of ECA was supplied from vertebral artery via Richter's anastomosis. Short arrow—cephalad direction.

were negative in all seven patients. In five patients the ICA stump pressure was less than 50 mm Hg. In four of these, the regional blood flow volume measured by SPECT was decreased during the BMT. Whenever the flow direction in the ICA was reverse, from the ECA to the ICA (cases 1, 3, and 7), the ICA stump pressure was less than 50 mm Hg and the regional blood flow volume measured by SPECT was decreased (Table 3). Reconstruction of the carotid artery was considered necessary in patients with ICA stump pressures of less than 50 mm Hg. The relative flow in the MCA during the CDMT correlated significantly with the ICA stump pressure during the BMT (Fig 3).

DISCUSSION

Clinical Application of Transcranial Doppler. Aaslid et al[10] measured the flow velocities in the MCA, anterior cerebral artery, and posterior cerebral artery, using the transcranial Doppler method (2 MHz). Padayachee et al[11] used the method in combination with CCA compression and evaluated the qualitative function of the circle of Willis. Recently, color Doppler imaging of intracranial vessels in young adults using a 2.25 MHz Doppler transducer has been reported.[12] However, we have not seen any reports evaluating the real-time quantitative function of the circle of Willis by measuring the blood flow velocity and volume in the MCA and the carotid bifurcation during Matas' test.

Increase of Blood Flow Volume in Contralateral CCA, ICA, and Bilateral VAs. Kaneko et al[13] reported increases in blood flow volume in the contralateral ICA and the bilateral VAs ranging from 20% to 40% during the unilateral CCA compression test, using the Doppler method. In this report the increases were 29.7% in the contralateral ICA, 50.4% in the ipsilateral VA, 42.8% in the contralateral VA, and 40.6% in the contralateral CCA. The blood flow volumes in the ICA and the VA were 323 and 154 mL/min, respectively, at rest.[14] From these data it was calculated that the increased blood flow volume in the other three vessels to the brain was 225 mL/min during unilateral CCA compression. This was 100 mL/min less than the flow volume in the ICA. Because these were the data for a 60-second period and did not include collateral flow to the brain via the ECA, careful evaluation is necessary.

Hemodynamics at Carotid Bifurcation During Matas' Test. Tindall et al[15] investigated the hemodynamics at the carotid bifurcation following occlusion of the CCA, using an electromagnetic flowmeter. They described flow reversal from the ICA to the ECA in all cases with good cross-filling via the anterior communicating artery (A-comm), but the flow direction was forward, from the ECA to the ICA, in all cases with poor cross-filling.

In this study there was no relationship between the relative flow volume of the MCA, the blood flow velocity, and/or the volume in the ICA during Matas' test, as shown in Table 2. This result is quite different from that of Tindall et al.[15] The difference is due to

TABLE 3. COMPARISON OF COLOR DOPPLER–GUIDED MATAS' TEST AND BALLOON MATAS' TEST

			Color Doppler–Guided Matas' Test			*Balloon Matas' Test*					
Case	*Age*	*Sex*	*MCA Flow (%)*	*Group**	*Flow Direction*	*Mean Stump Pressure of ICA (mm Hg)*	*SPECT (Laterality)*	*A-Comm Cross Flow*	*EEG*	*Neurologic Signs*	*Operation*
1	48	M	79	B	Forward	35	+	Good	Normal	-	Bypass
2	52	M	63	C	Reverse	35	+	Good	Normal	-	Bypass
3	46	M	78	B	Reverse, then forward	42	+	Good	Normal	-	Bypass
4	57	M	85	B	Reverse	56	-	Good	Normal	-	Abrupt resection of ICA and CCA
5	67	M	83	B	Reverse	49	-	Good	Normal	-	ND
6	51	M	93	A	Reverse	86	-	Good	Normal	-	ND
7	62	M	63	C	Reverse, then forward	47	+	Good	Normal	-	Bypass

MCA — middle cerebral artery, ICA — internal carotid artery, SPECT — single photon emission computed tomography, A-comm — anterior communicating artery, EEG — electroencephalogram, CCA — common carotid artery, ND — not done.

*Defined in text.

their method, in that 1) they did not measure the ICA flow directly, 2) they drew their conclusions from blood flow direction and volume in the ECA only, disregarding Richter's collateral circulation, and 3) the duration of occlusion in the CCA was as short as 30 seconds.

Evaluation of Brain Collateral Circulation and Indications for Reconstruction of Carotid Artery. To determine whether reconstruction of the carotid artery is indicated, it is necessary to investigate the brain's collateral circulation in the circle of Willis. Matsuda et al[2] evaluated the cerebral collateral circulation by technetium 99m hexamethyl-propyleneamine oxime brain SPECT during Matas' test. They concluded a critical point could exist somewhere between a 19% and a 29% reduction in radioactivity; that is, between a 27% and a 38% reduction in blood flow volume.

Yoshida and Ishijima[3] measured regional cerebral blood flow with xenon 133 during Matas' test. They concluded that ligation of the CA should not be performed in cases in which there is a flow of less than 25 to 30 mL/100 g of brain per minute. Therefore, a critical point could exist between 60% and 75% of the pretest flow.

Atkinson et al[4] used the ICA stump pressure and EEG during Matas' test for more accurate estimation. They concluded that a stump pressure of less than 50 mm Hg and having more than a 50% attenuation of the somatosensory-evoked cortical potential were indications for reconstruction of the carotid artery.

Leech et al[16] measured regional cerebral blood flow during Matas' test by using xenon 133 and decided that ligation of the CCA could be performed when there was less than a 25% reduction in radioactivity, even if the ICA stump pressure increased, but that it was necessary for there to be an increase of more than 60 mm Hg in stump pressure in cases in which there was between a 25% and a 35% reduction in radioactivity.

The reason for combining methods is to compensate for the defects in individual methods: the absolute value of the stump pressure is affected by systemic blood pressure, the EEG cannot evaluate brain flow volume directly, and SPECT is not a real-time procedure and cannot measure blood flow volume in particular vessels.

Our method can measure the blood flow volume and velocity for particular vessels, and estimate the

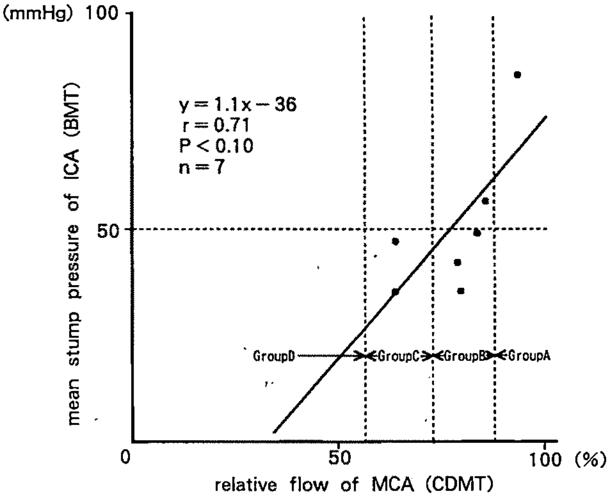

Fig 3. Correction between relative flow of middle cerebral artery (MCA) on color Doppler–guided Matas' test (CDMT) and mean stump pressure of internal carotid artery (ICA) on balloon Matas' test (BMT). Relative flow of MCA during CDMT was significantly correlated with ICA stump pressure during BMT.

brain's collateral circulation on the basis of relative flow change in the MCA. Our data are not affected by systemic blood pressure. The incidence of complications of CCA ligation was 26% to 30.1% in transitory cerebral symptoms, and there was a 13% to 15.4% mortality rate.[17-19] On the other hand, it was reported that there were no cerebral complications in 57.4% of cases with abrupt CCA occlusion. These data suggest a critical point in brain blood flow volume at between 60% and 75% of the blood flow volume before a Matas' test.

These papers and results indicate that the brain collateral circulation is very good in group A; fair (and therefore it is possible to abruptly ligate the CCA) in group B; slightly poor (with risks of cerebral complications) in group C; and poor (meaning there is the risk of death with ligation of the carotid artery) in group D.

When there is reversal of flow at the carotid bifurcation during Matas' test, ligation of the ICA increases the blood flow to the brain. Considering the increase in the flow in the bilateral VAs and the contralateral ICA, abrupt ligation of the CCA could be possible without reconstruction of the carotid artery. Conversely, when there is forward flow from the ECA to the ICA at the carotid bifurcation during Matas' test, ligation of the ICA occludes the collateral circulation in the neck and carries a greater risk of cerebral ischemia than with ligation of the CCA, and reconstruction of the carotid artery should be performed.

Konno et al[18] reported that the incidence of complications was proportionally lower in the group with intact ECAs than in the group with ligated ECAs. From the point of view of the brain blood flow volume, their finding is due to the collateral circulation from the ECA to the ICA. Regarding propagation of thrombus, there are two types. One type has a drainage route with an intact ECA, while the other type does not have a drainage route but has a ligated ECA and a site of thrombus formation. The former has a lower risk of cerebral infarction. In groups B and C, further examinations (balloon Matas' test, SPECT, and brain angiography) should be performed. Reconstruction of the carotid artery needs to be considered. In group D, reconstruction of the carotid artery should always be performed.

The balloon CDMT with the CCA and ICA occlusion test could be used to evaluate the collateral circulation more accurately. The relative flow of the MCA during the CDMT was significantly correlated with the ICA stump pressure and SPECT during the BMT. The CDMT test is a very useful examination for estimating brain collateral circulation because it is a noninvasive, real-time, and repeatable procedure.

Indications for reconstruction of the carotid artery can be decided on the basis of this method. An improvement in the survival rate of advanced metastatic neck tumor can be expected with radical surgery including resection of the carotid artery with or without reconstruction.

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CELL KINETICS STUDY OF UPPER AERODIGESTIVE TRACT SQUAMOUS CELL CARCINOMA USING BROMODEOXYURIDINE

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Thirty patients treated for squamous cell carcinoma of the upper aerodigestive tract were reviewed for a cell kinetics study using bromodeoxyuridine (BrdU). Fresh specimens were obtained from the primary site of the tumors and incubated in Dulbecco's modified Eagle's medium containing BrdU to label *S*-phase tumor cells. The tumor specimens were stained immunohistochemically with monoclonal antibody against BrdU. The results demonstrated that the BrdU labeling index (BrdU LI) in these cases (470.3 ± 118.2) was greater than the BrdU LI in normal mucosa (162 ± 52.4). In a comparison of the clinical data and histopathologic features, the BrdU LI showed an inverse correlation with patient age ($r = -.558$; $p < .01$) and a direct correlation with the frequency of mitoses ($r = .411$; $p < .05$). Although the significance of the BrdU LI as a prognostic factor cannot be determined at present, this technique provides a simple in vitro test that avoids the potential hazards of in vivo labeling or the use of radioactive materials.

KEY WORDS — **bromodeoxyuridine, squamous cell carcinoma, upper aerodigestive tract.**

INTRODUCTION

Studies of growth kinetics of tumor cells that quantify the proliferation rate are important for measuring the effect of therapy and as a prognostic indicator. [^{3}H]thymidine incorporation into DNA, detected by autoradiography, has been widely applied in cell kinetics studies of malignant tumors in vivo and in vitro, but its use has been limited by its potentially hazardous radioactivity. Bromodeoxyuridine (BrdU) is a nonradioactive analogue of thymidine, is incorporated into DNA of cells in *S*-phase DNA, and can be detected following incorporation by using anti-BrdU antibodies. This method is more convenient for the investigation of cell kinetics than autoradiography using [^{3}H]thymidine.[1,2] In this study, tumor tissues and normal mucosa from patients with squamous cell carcinoma were cultured in a medium containing BrdU for 2 hours. Incorporation of BrdU into cells was detected immunohistochemically by using a monoclonal antibody against BrdU. The proportion of DNA-synthesizing cells (BrdU labeling index [BrdU LI]) was measured microscopically and compared with clinical data and the histology of the tumor.

MATERIALS AND METHODS

A total of 31 squamous cell carcinomas arising in the mucosa of the upper aerodigestive tract derived from 30 patients were investigated. Normal mucosa was examined in 12 patients as a control. A 5-mm^3 fragment of fresh specimen was removed from the primary site and cut into sections 0.5 mm in thickness, and transferred into culture vessels containing 100 µmol/L BrdU in Dulbecco's modified Eagle's medium and 10% fetal calf serum. Then 95% oxygen, 5% carbon dioxide gas was introduced into the culture vessels at three times atmospheric pressure. The tissues were incubated at 37°C in a gyratory shaker water bath at 100 strokes per minute for 2 hours. The specimens were then fixed in 4% buffered formol-saline for 24 hours and embedded in paraffin.

Following cutting of 5-µm thin sections and deparaffinization, the sections were treated with 0.1% trypsin and 0.1% calcium chloride (pH 7.7) for 30 minutes at 37°C. After thorough washing with phosphate-buffered saline (PBS; 0.15 mol/L sodium chloride in 0.01 mol/L phosphate buffer, pH 7.2), all sections were incubated in 5% nonfat dry milk containing 0.5% Nonidet P-40 and 0.01% sodium azide for 30 minutes at room temperature to block nonspecific binding. After washing with PBS, the sections were treated with 4N hydrochloric acid for 20 minutes to denature DNA. Sections were rinsed five times in distilled water, followed with three washes in PBS. They were reacted with primary monoclonal antibody against BrdU (Dakopatts) at a 1:80 dilution for 30 minutes in a moist chamber. The tissues were then rinsed three times with PBS and reacted with biotin-labeled horse anti mouse immunoglobulin G (Vector Labs) for 30 minutes. After washing three

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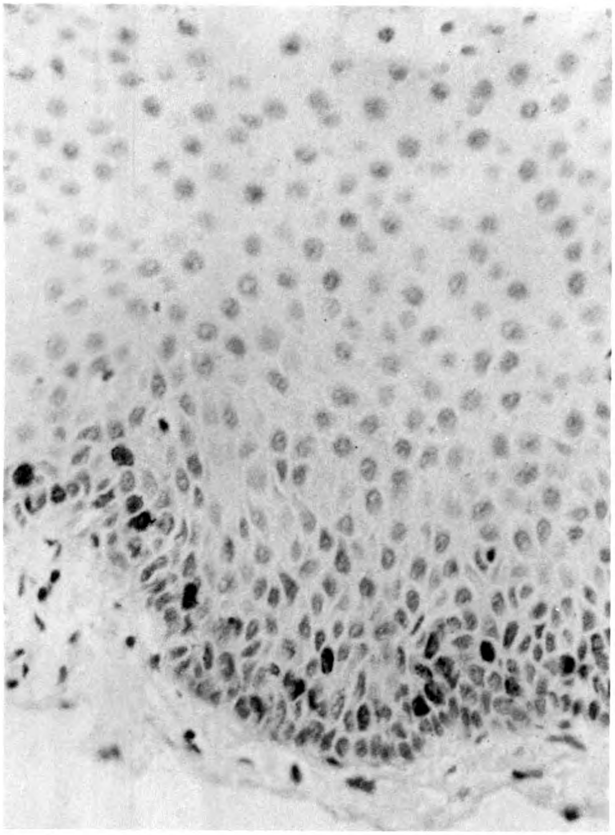

Fig 1. Normal pharyngeal mucosa from 60-year-old man (original x360). Positive immunoreactivity is seen in basal cell layer. Bromodeoxyuridine labeling index of this mucosa is 144 to 2,000.

times with PBS, the endogenous peroxidase activity was blocked by a 15-minute incubation in methanol containing 0.3% hydrogen peroxide. The slides were rinsed with PBS and the tissue overlaid with a peroxidase-avidin conjugate for 30 minutes. Following washing in PBS, the sections were immersed in a reagent — 0.02% 3,3-diaminobenzidine tetrahydrochloride, 0.005% hydrogen peroxide, and 0.05 mol/L TRIS buffer, pH 7.6 — for 10 minutes, counterstained with Harris' hematoxylin, dehydrated in ethanol, cleared in xylene, and coverslipped by means of Permount.

In addition to use of primary monoclonal antibody, a negative control was performed without the application of primary antibody, and these sections all showed no reaction. The labeling index (LI; BrdU-positive cell nucleus) was obtained after counting 2,000 tumor cells or normal epithelial cells under a light microscope. Statistical significance was evaluated by means of Student's *t* test.

Thirty patients with squamous cell carcinoma were entered in this study: 21 were men and 9 were women, with age at diagnosis ranging from 44 to 78 years old. The primary sites of squamous cell carcinoma were oral cavity (14 patients), oropharynx (6), tonsil (5), larynx (4), and ear (1). One patient with oral cavity squamous cell carcinoma had both the primary tumor and a recurrence evaluated. The tumors were staged according to the guidelines of the American Joint Committee for Cancer Staging and End Results Reporting (1989): none were stage I, 4 were stage II, 4 were stage III, and 23 were stage IV. Histologic evaluation of tissues was based on Jakobsson's grading scheme as modified by Crissman et al.[3]

RESULTS

Labeling Index in Normal Tissue and Squamous Cell Carcinoma. The nuclei that incorporated BrdU were clearly stained by the immunohistochemical method, and it was easy to distinguish BrdU-labeled nuclei from unlabeled ones. Normal stratified squamous epithelium showed a positive immunoreactivity in the basal cell layer (Fig 1). On the other hand, tumor tissues revealed positivity in a mosaic pattern (Fig 2).

The BrdU LI was determined in 12 specimens of normal tissue from 12 patients and the 31 squamous cell carcinomas from 30 patients. The mean BrdU LI (±SD) in the 12 normal tissues was 162 ± 52.4 per 2,000 cells. The mean BrdU LI in the 31 squamous cell carcinoma tissues was 470.3 ± 118.2. Squamous cell carcinoma tissues showed a significantly higher BrdU LI ($p < .01$) than normal tissues by Student's *t* test.

Labeling Index and Clinical Data. The BrdU LI of squamous cell carcinoma and patient age showed a statistically significant inverse correlation, with the coefficient of correlation (r) being −.558 ($p < .01$). The mean BrdU LI (±SD) was 460.9 ± 117.1 in men, and the mean BrdU LI was 493.0 ± 125.0 in women. Therefore, there was no significant difference by sex.

There was also no significant difference by primary tumor sites: oral cavity 448.4 ± 131.6, larynx 447.0 ± 121.7, oropharynx 543.5 ± 118.0, tonsil 475.0 ± 69.9, and ear 427.0. There was no significant difference by T classification: T1 426.5 ± 36.1, T2 485.7 ± 92.7, T3 510.2 ± 154.5, T4 490.8 ± 123.2, and TX 418.4 ± 127.6. There was no statistically significant difference by staging: stage II 531.8 ± 26.6, stage III 505.0 ± 121.2, and stage IV 435.5 ± 125.6.

Labeling Index and Histology. Cytoplasmic keratinization was graded with a score of 1 to 4. The mean BrdU LI for each score was as follows: score 1 365.0 ± 161.8, score 2 494.5 ± 118.5, score 3 489.6 ± 48.7, and score 4 491.0 ± 152.4. There were no statistically significant differences among them.

Nuclear differentiation was scored according to the Table,[3] with no patient having a score of 1. The

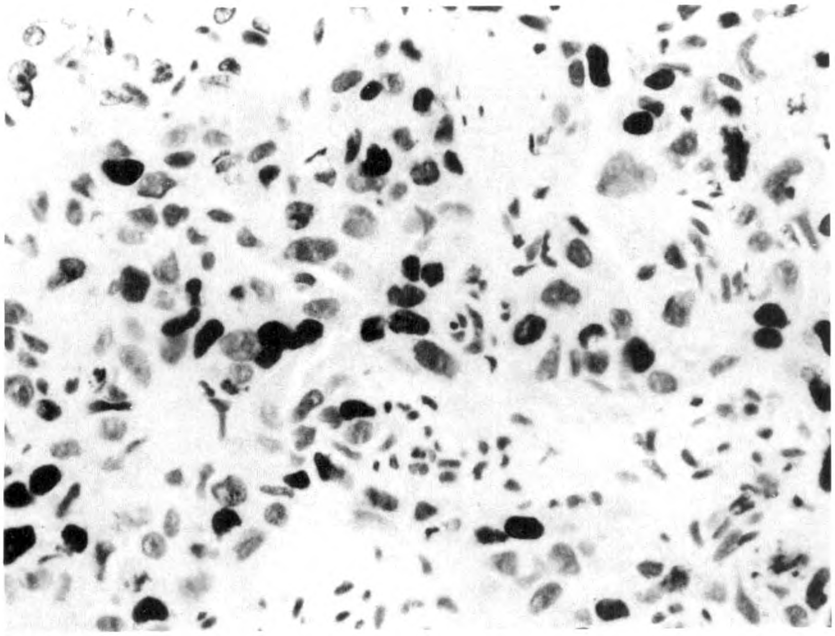

Fig 2. Squamous cell carcinoma (T3N3M0) derived from posterior hypopharyngeal wall of 49-year-old woman (original x360). Many labeled (dark) atypical cells are seen in tumor tissue. Bromodeoxyuridine labeling index of this case is 670 to 2,000.

mean BrdU LI for each score was as follows: score 2 491.6 ± 111.6, score 3 444.2 ± 133.4, and score 4 495.5 ± 104.5. There were no statistically significant differences.

The average number of mitoses per high-power field was assigned to one of the four groups referred to in the Table. The mean BrdU LI for each score was as follows: score 1 393.0 ± 131.7, score 2 461.4 ± 104.8, score 3 494.9 ± 114.7, and score 4 548.2 ± 101.2. There were no statistically significant differences among them, but the coefficient of correlation (r) was .411, and the grading of the average number of mitoses per high-power field and BrdU LI showed a significant correlation ($p < .05$).

The patterns of invasion were classified into four groups according to the Table, with no patient having a score of 1. The BrdU LI for each score was as follows: score 2 447.4 ± 79.7, score 3 450.2 ± 143.7, and score 4 512.8 ± 99.0. There were no statistically significant differences.

Lymph node metastasis was determined histologically. The mean BrdU LI of tumors with metastasis was 493.5 ± 127.9, and the mean BrdU LI of tumors without metastasis was 463.7 ± 111.3. There was no statistically significant difference.

Vascular invasion was evaluated and correlated with BrdU LI scores. The positive group demonstrated a score of 495.7 ± 111.7, and the negative group, 429.0 ± 120.2. There was no statistically significant difference.

DISCUSSION

Several prognostic factors for squamous cell carcinoma in the upper aerodigestive tract have been evaluated. The size and site of the primary tumor and

HISTOPATHOLOGIC GRADING SCHEME FOR SQUAMOUS CELL CARCINOMA

	Score			
Histologic Parameter	*1*	2	3	4
Cytoplasmic keratinization	High degree, well-formed pearls	Moderate, 20% to 50% of cells, attempt at pearl formation	Poor, 5% to 20% of cells with suggestion of keratinization	No evidence of keratinization
Nuclear differentiation	Few enlarged nuclei, >75% mature-appearing	50% to 75% mature-appearing nuclei	Considerable nuclear pleomorphism, 25% mature-appearing	Anaplastic tumor
Average number of mitoses per high-power field	0-1	2-3	4-5	>5
Inflammatory response	Continuous rim	Patchy rim	Occasional patch	None
Vascular or lymphatic invasion	Not identified	Not identified	Not identified	Identified
Pattern of invasion	Pushing borders	Solid cords	Thin, irregular cords	Single cells

Information from Crissman et al.[3]

the presence of metastatic nodal disease,[4] histologic criteria of tumor aggressiveness,[3] and tumor thickness have all been shown to correlate with survival.[5,6] These guidelines and grading systems, however, have shown poor correlation with clinical outcome in some patients.

The cell kinetics study of human malignant tumors may be able to produce a better understanding of the aggressiveness of the tumor, therapeutic effects, and prognosis. Many studies using [^{3}H]thymidine have been performed, but this method uses radioactive materials and is time-consuming.

Using BrdU, we have investigated the cell kinetics of human squamous cell carcinomas and normal tissues by using short culture times and immunohistochemical staining. Bromodeoxyuridine is a pyrimidine analogue of thymidine that is incorporated into DNA during the *S* phase, and cells containing BrdU in their DNA can be detected with a monoclonal antibody against BrdU. Target tissues may be labeled with BrdU either in vivo or in vitro. We believe that in vitro BrdU labeling is an attractive technique, considering the potential hazards of in vivo labeling.[7,8] Several reports are available on the cell kinetics of human malignant tumors studied with [^{3}H]thymidine autoradiography and BrdU.[9-11] They have reported that LI by autoradiography and LI by BrdU are closely correlated. Because it is less time-consuming and does not use radioactive material, BrdU is thought to be a much better label than [^{3}H]thymidine.[1,2] The correlation between BrdU LI and percentage of *S*-phase cells as measured by flow cytometric measurement was also reported previously in breast carcinoma.[12]

Shimosato et al[13] demonstrated the BrdU LI of lung adenocarcinoma to range from 0.1% to 19.2%, with a mean of 3.6%, by in vitro labeling; that in lung squamous cell carcinoma ranged from 7.8% to 15.4%, with a mean of 11.0%. Kikuyama et al[14] demonstrated 4.0% to 41.4% in gastric carcinoma, and Germano et al[15] reported 0% to 38.2% in gliomas by in vivo labeling. Our results showed that squamous cell carcinoma has a mean BrdU LI of 24.4% (487 per 2,000), ranging from 9.2% to 33.5%, and this figure is statistically significant as compared to the mean BrdU LI of normal epithelium. The discrepancy among individual tumors might be caused by differences in labeling technique, incubation time, BrdU concentration, or the primary site. Our results demonstrated that the mitotic index correlated well with the BrdU LI ($r = .411$), and this fact supports the report of Shimosato et al.[13] Crissman et al[3] have reported that the frequency of mitoses is an important predictor of survival. Germano et al,[15] however, reported no correlation between the BrdU LI and mitotic index in brain tumors. On the other hand, our results showed an inverse correlation between the BrdU LI and patient age ($r = .539$), with the tumors found in the younger patients having a higher BrdU LI than the tumors in the older patients. The significance of this is obscure. Unlike Shimosato et al,[13] who reported a high mean index in adenocarcinomas with marked atypia and in larger tumors, our results show no significant correlation between the LI and either tumor size or nuclear differentiation. This discrepancy might reflect the difference in clinicopathologic characteristics between squamous cell carcinoma and adenocarcinoma.

In the one case in which both the primary tumor and a locally recurrent tumor were examined, the BrdU LI showed an increase from 428 in the primary to 536 in the recurrent tumor. Although this is noteworthy, more cases of recurrent tumor must be examined in order to determine the significance of this phenomenon. Moreover, the therapeutic effect could be examined by comparing BrdU LIs before and after therapy.

Cell kinetics using BrdU is considered to be a possibly important prognostic factor, particularly in malignant tumors.[1] The cases reported are derived from patients seen in the past 12 months, and therefore the value of BrdU as a prognostic factor cannot be determined at present. However, this in vitro method of determination of cellular proliferation is simpler and more rapid than use of [^{3}H]thymidine, and without the potential for radiation hazard, and it may prove useful for further studies in this field.

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MUCOSAL FOLDS IN HUMAN EUSTACHIAN TUBE: A HYPOTHESIS REGARDING FUNCTIONAL LOCALIZATION IN THE TUBE

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Differences in the extent of mucosal folding in the superior versus the inferior half of the human eustachian tube (ET) lumen were studied by measuring, as it appeared in histologic cross section, the length of the mucosal margin in the pharyngeal portion, the midcartilaginous portion, the isthmus portion, and the osseous portion of each half of the ET in 16 normal ET temporal bone specimens from 16 individuals 12 to 70 years of age. The mucosal margin of the ET was significantly longer in the inferior half than in the superior half in all four portions (paired t test: pharyngeal portion $t[15] = 3.59$, $p < .01$; midcartilaginous portion $t[15] = 3.52$, $p < .01$; isthmus portion $t[15] = 4.15$, $p < .01$; osseous portion $t[15] = 2.24$, $p < .05$). These results indicate that the inferior half of the ET mucosa has more folds than the superior half, and that the surface area of the inferior half of the ET is larger than that of the superior half. This, together with the finding previously reported that secretory components of the ET such as glands and goblet cells are more richly distributed in the inferior (floor) portion than in the superior (roof) portion of the ET, may be related to the fact that the floor of the ET has an active role in clearance function. A hypothesis regarding localization of the ventilatory and clearance functions within the ET is proposed.

KEY WORDS — eustachian tube, middle ear clearance, middle ear ventilation, mucosal fold.

INTRODUCTION

The eustachian tube (ET) has three major functions: ventilation, clearance, and protection of the middle ear (ME).[1] Of these three, the first two functions appear to be of particular significance, because they are active functions of the ET. We have investigated these functions of the ET morphologically by studying a large number of human ET and temporal bone specimens (specimens that were harvested, by the technique we developed,[2] to include the entire ET and its surrounding structures), and recently we have come to advocate a hypothesis that these different functions are localized within the ET. Thus, we believe that the superior (roof) portion of the ET may be mainly involved with the ventilatory function, whereas the inferior (floor) portion may be mainly involved with the clearance function.

In one of our previous studies, we found that the secretory components of the ET — its glands and goblet cells — are distributed more richly in the floor portion than in the roof portion of the ET.[3] This finding favors the part of our hypothesis that states that the floor portion of the ET may be mainly involved with the clearance function. Another anatomic feature of the ET that we believe is relevant to its clearance function is its mucosal folds. That these folds are more richly distributed in the floor portion of the ET has been pointed out by several investigators,[4,5] but to our knowledge no quantitative analysis has been done to confirm this. Thus, we conducted

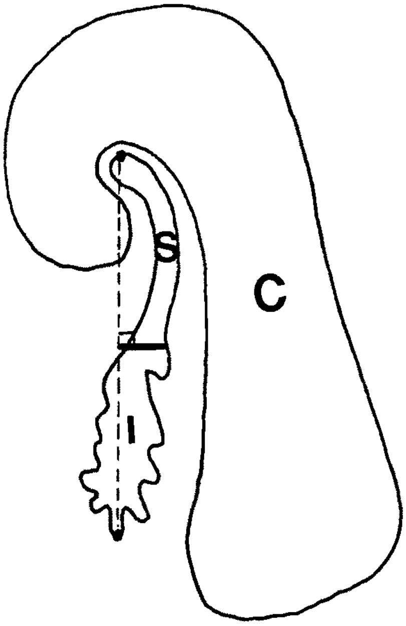

Fig 1. Schematic representation of method for determining upper and lower mucosal margins on cross-sectional portion of human eustachian tube. Dashed line connects most superior and inferior points, and solid line separates superior (S) and inferior (I) halves of tube. C — eustachian tube cartilage.

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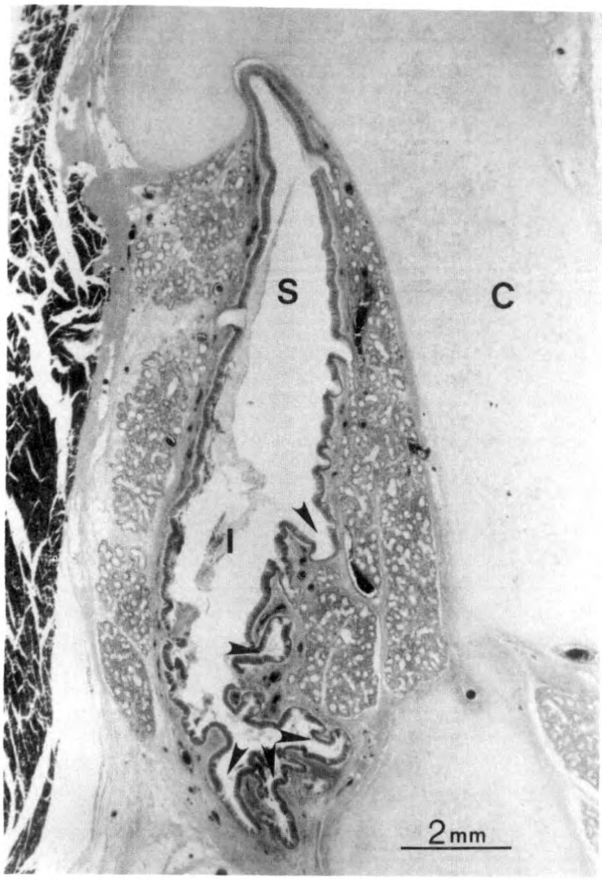

Fig 2. Photomicrograph of cross section through midcartilaginous portion of eustachian tube of 19-year-old woman (H & E). Mucosal folds (arrowheads) are more abundant in inferior (I) half of tube than in superior (S) half. C — eustachian tube cartilage.

the study reported here, in which we compared the extent of mucosal folding in the superior and inferior halves of the ET as evidenced on histologic cross sections.

METHODS AND MATERIALS

Sixteen normal human temporal bone specimens with the entire ET and its accessory structures, obtained from 16 individuals 12 to 70 years of age, were studied. All specimens were processed histologically according to the technique described by Sando et al.[2] Serial 30-μm-thick histology sections were cut vertically in the plane perpendicular to the long axis of the ET, and every 20th section was stained with hematoxylin and eosin. Sections from the four portions (pharyngeal portion, midcartilaginous portion, isthmus portion, and osseous portion) of each ET were projected onto paper, and the margin of the ET lumen was traced. As shown in Fig 1, the ET was divided into superior and inferior halves by drawing a line through the midpoint of and perpendicular to a line connecting the most superior and inferior points on the ET margin. The lengths of the mucosal margins of the superior and inferior halves in each portion of the ET were then measured with a digitizer (Hitachi HDG-1515) and compared for each ET.

RESULTS

A photomicrograph of a representative histologic section through the midcartilaginous portion of the ET is shown in Fig 2. More abundant mucosal folds may be seen in the inferior half of this ET than in its superior half. In this particular case, the mucosal margin in the inferior half of the ET (12.71 mm) was far longer than that in the superior half (6.50 mm). This relationship was true for most cases in this study for all four portions of the tube examined (paired t test: pharyngeal portion $t[15] = 3.59$, $p < .01$; midcartilaginous portion $t[15] = 3.52$, $p < .01$; isthmus portion $t[15] = 4.15$, $p < .01$; osseous portion $t[15] = 2.24$, $p < .05$; Fig 3 and Table).

DISCUSSION

The finding of the present study that the mucosal margin in the inferior half of the ET was longer than that in its superior half in all four portions examined indicates that there is greater mucosal folding in the inferior half of the ET.

The finding of rich mucosal folding in the floor of the ET, together with previous findings of richer populations of ciliated cells,[6] glands,[3] and goblet cells[3] (Fig 4) in the floor versus the roof of the ET, points to more active clearance taking place in the floor portion of the ET than in its roof. This increased folding has two potential consequences. First, since mucosal folding enlarges the mucosal surface area of the narrow tubal lumen, it increases the available area for ET clearance. Second, when the ET opens to ventilate the ME, the airflow proceeds in the tubal lumen from the nasopharynx toward the ME. By contrast, the smooth transportation of secretions proceeds in the opposite direction, from the ME toward the nasopharynx. Thus, mucosal folding of the ET floor may isolate the site of transport from the airflow.

Ventilation of the ME is well known to occur by active opening of the ET, mediated for the most part by contraction of the tensor veli palatini muscle (TVPM).[7,8] To investigate our hypothesis that the roof portion of the ET may be mainly involved with the tube's ventilatory function, we conducted several studies,[9-11] with the following results. In normal individuals, the TVPM inserts into the tip of the lateral lamina (LL) of the ET cartilage in its midcartilaginous portion,[9] as shown in Fig 5. This fact, and the observation that the TVPM courses at nearly a right angle to its insertion site on the LL,

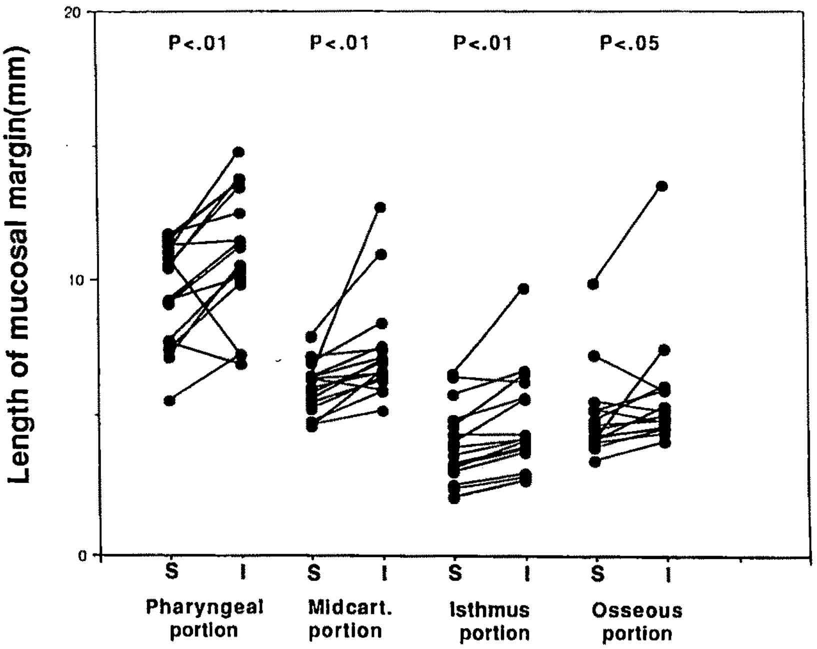

Fig 3. Graphic representation of lengths of mucosal margin in superior (S) versus inferior (I) halves in four portions of eustachian tubes from 16 individuals. Midcart. portion — midcartilaginous portion.

seem to indicate that contraction of the TVPM moves the LL inferolaterally away from the medial lamina in a hinge action.[12] Such an action would be an efficient mechanism for active opening of the ET. When the LL is well developed, as it is in normal individuals, it covers the roof portion of the tubal lumen[10] laterally so that the roof portion of the tube is mostly surrounded by cartilage. Also, in normal individuals with a well-developed LL, the tubal lumen was found to be C-shaped, or concave to the LL, in cross section.[10] This concavity of the tubal lumen would appear to allow for a notable amount of tubal expansion laterally, with inferolateral movement of the LL consequent to contraction of the TVPM — thus making smooth active opening of the ET (especially its upper rotation) easier (Fig 6). On the other hand, the floor portion of the ET may be resistant to opening, because it is surrounded by gland tissue and separated from the TVPM by Ostmann's fatty tissue.

We also observed a rich, meshlike distribution of elastin on the luminal side of the intermediate portion (hinge portion) of the tubal cartilage, between the medial lamina and LL, in normal individuals[11] (Fig 5). The presence of this elastin, first pointed out by Guild,[13] led us to speculate that the LL, after moving by contraction of the TVPM away from the medial lamina, may return to its original position as a result

LENGTH OF MUCOSAL MARGIN IN MILLIMETERS

		Pharyngeal Portion		*Midcartilaginous Portion*		*Isthmus Portion*		*Osseous Portion*	
Age	*Sex*	*S*	*I*	*S*	*I*	*S*	*I*	*S*	*I*
70	M	7.46	9.82	7.02	8.44	2.91	3.63	3.35	4.05
68	M	9.08	11.22	7.23	7.46	6.48	6.27	3.84	4.56
69	M	11.41	13.57	6.40	5.97	3.23	3.82	5.55	5.22
34	M	10.53	13.39	7.97	10.93	6.57	9.73	4.84	6.15
17	F	5.58	7.32	6.06	6.67	3.54	4.15	5.20	5.96
29	F	9.22	10.05	5.65	7.14	3.83	4.12	7.30	5.96
26	M	10.40	13.80	5.87	6.98	2.44	2.85	4.68	4.87
52	F	11.03	14.81	6.49	7.62	5.82	6.70	5.26	4.80
12	M	11.68	12.48	5.49	6.33	3.06	4.09	4.49	5.02
22	F	7.71	6.94	5.29	6.45	4.59	6.59	4.10	5.39
49	M	9.25	11.39	4.66	5.23	3.15	3.77	4.24	4.56
28	F	7.76	10.32	6.48	7.19	2.31	2.75	4.01	4.38
19	F	11.23	11.41	6.50	12.71	4.03	5.60	4.10	7.56
38	F	7.19	10.51	6.43	6.58	4.83	5.67	9.88	13.51
18	M	10.77	7.31	4.59	6.69	2.02	2.61	4.22	4.61
30	M	11.57	13.61	4.79	5.91	4.33	4.34	4.97	4.94

S — superior half, I — inferior half.

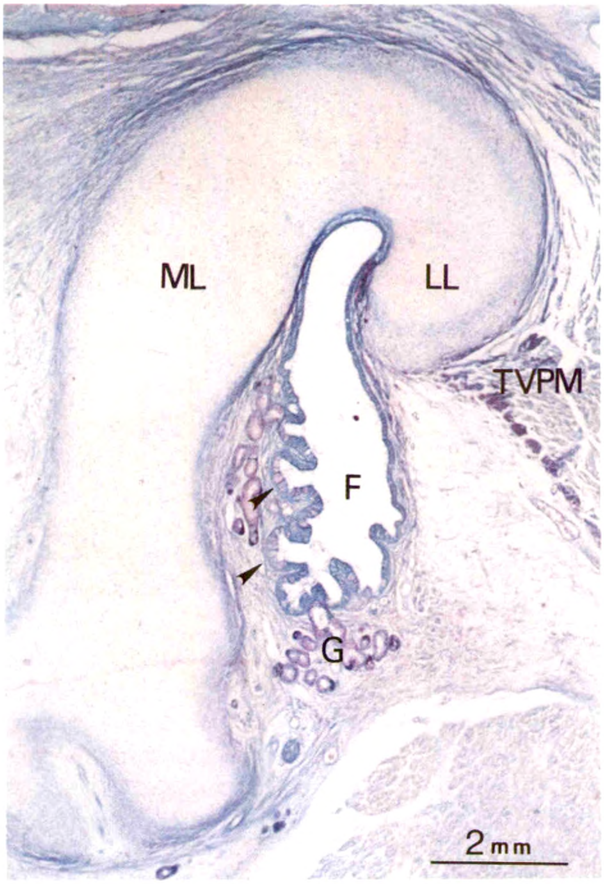

Fig 4. Photomicrograph of cross section through midcartilaginous portion of human eustachian tube. Staining with periodic acid–Schiff–Alcian blue and hematoxylin demonstrates goblet cells (arrowheads) in tube and tubal glands (G) near floor (F) of tube. LL — lateral lamina, ML — medial lamina, TVPM — tensor veli palatini muscle.

of elastic action at this point (Fig 6). Taken together, the findings in structures surrounding the ET, especially its roof portion, point to the possibility that active opening of the ET for ventilation of the ME is mainly a function of inferolateral movement of the LL and the roof portion of the ET. Indirect support for the importance of these anatomic characteristics in ventilating the ME is provided by the finding that they are defective in children with cleft palate[9-11] and that ventilatory dysfunction and otitis media are frequently seen in children with this anomaly.[14,15]

The histologically documented anatomic characteristics of the ET important to our hypothesis are summarized here. That the roof portion is important in ventilation is supported by the following findings.

1. Consistent insertion of the TVPM at a right angle to the tip of the LL of the tubal cartilage.
2. Complete development of the LL to produce a concave cross section of the tubal lumen.
3. Curvature of the tubal lumen.
4. Rich distribution of elastin at the hinge portion of the tubal cartilage.

That the floor portion is important in clearance is supported by the following findings.

1. Rich distribution of glands and goblet cells.
2. Abundant mucosal folds.

Our hypothesis that the upper part or roof of the ET serves the ventilatory function and that the floor serves for clearance is reasonable, in that air, with a lower specific gravity than mucus, would be expected to pass through the upper part of the ET, while heavier fluid or mucus would be expected to travel more inferiorly.

Finally, we would like to investigate in greater

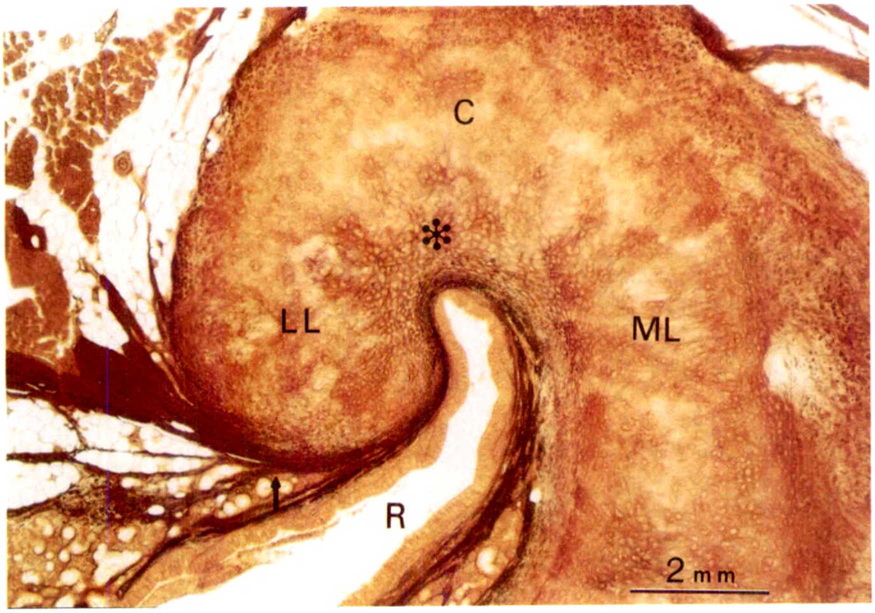

Fig 5. Cross section through midcartilaginous portion of eustachian tube stained with Weigert's elastin stain. Note rich, meshlike distribution of elastin (asterisk) along luminal side of intermediate portion between medial lamina (ML) and lateral lamina (LL) of tubal cartilage (C). Arrow indicates tendon of tensor veli palatini muscle attaching to tip of LL. Also note that roof portion (R) of tube is concave in cross section relative to LL.

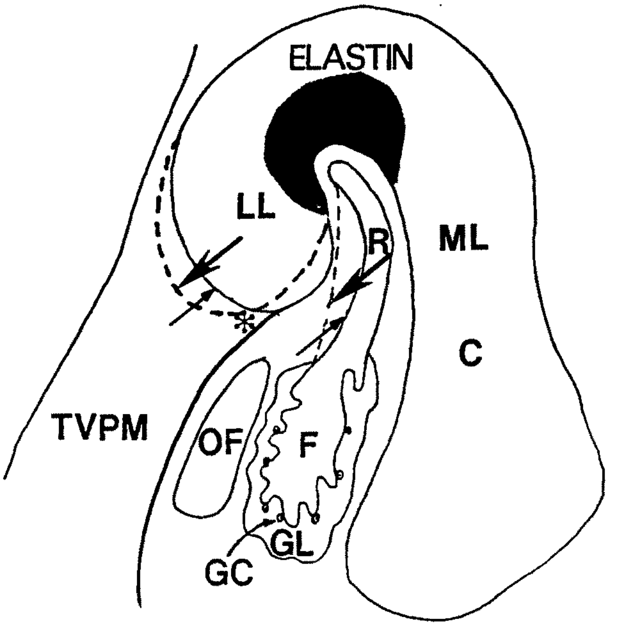

Fig 6. Schematic drawing of eustachian tube (midcartilaginous portion) in cross section, showing anatomic characteristics important in functions of tube. Thick arrows indicate direction of movement of lateral lamina (LL) of tubal cartilage (C) and of consequent movement of upper lateral epithelial wall of roof portion (R) of eustachian tube when tensor veli palatini muscle (TVPM) contracts. Dashed lines indicate location of LL and upper part of lateral epithelial wall of tube after TVPM has contracted. Note that there is no particular structure between LL and R to hamper opening of tubal lumen, whereas expansion of floor portion (F) of tube is hindered by Ostmann's fatty tissue (OF) between TVPM and tube and by tubal glands (GL) surrounding tube. There is no traceable tissue connection between TVPM and tubal epithelium (personal observations). Thin arrows indicate return of LL and lateral epithelial wall, as result of elastin located in dark crosshatched area of C, to their resting positions when TVPM relaxes. These anatomic relationships and presence of GL, goblet cells (GC), and infolded epithelial lining of eustachian tube — mainly in F — suggest that R effects ventilation and that F performs clearance function. ML — medial lamina, asterisk — attachment of tendon of TVPM to C.

detail the three-dimensional relationship between Ostmann's fatty tissue and the ET. We believe that this relationship will be found to strengthen our hypothesis on functional localization of the ET.

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COMPARISON OF QUANTITATIVE DNA MEASUREMENTS AND CYTOMORPHOLOGY IN SQUAMOUS CELL CARCINOMAS OF THE UPPER AERODIGESTIVE TRACT WITH AND WITHOUT LYMPH NODE METASTASES

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Cytologic smears of squamous cell carcinomas (UICC T2 through T4) from the mucosa of the upper aerodigestive tract without cervical lymph node metastases (n = 10) and with metastases (n = 20) were examined. Subsequently, the cytologic smears of the metastases (n = 20) obtained by fine-needle aspiration or following neck dissection were evaluated morphologically. Each specimen was then stained with Papanicolaou and Feulgen techniques, and quantitative DNA measurements were performed with an image analysis system. Morphologically, the primary tumors with metastases revealed an increased mean nuclear perimeter and mean nuclear area compared with tumors unassociated with metastases. On quantitative DNA measurements, the tumors with metastases had an increased DNA content, a higher 2c deviation index, and a higher DNA malignancy grade and increased number of aneuploid cells. The specimens obtained from the metastatic nodes yielded the highest values for mean DNA content, 2c deviation index, aneuploidy, and malignancy grade. The stem lines of metastasis were always nondiploid. These data indicate a positive correlation between aneuploid tumor cell clones in primary cancer and the manifestation of lymph node metastases.

KEY WORDS — head and neck, lymph node metastases, primary tumor, quantitative DNA measurements, squamous cell carcinoma.

INTRODUCTION

Neither clinical stage nor histologic grading is a satisfactory prognostic indicator for squamous cell cancer of the mucosa of the upper aerodigestive tract. For squamous cell carcinomas, the depth and mode of invasion and the tumor size have to date proved to be the most helpful in predicting the development of lymph node (LN) metastases.[1-4]

Quantitative DNA measurements have demonstrated prognostic relevance in many tumors, eg, breast cancer,[5-10] prostatic cancer,[11-15] and malignant lymphoma.[16,17] For head and neck cancers, a significant correlation between DNA analysis results and survival was found in both laryngeal cancer and cancer of the oral cavity.[18-20]

The purpose of the present study is to examine the cytomorphologic data and to compare DNA analyses in patients with squamous cell carcinoma of the head and neck with and without LN metastasis.

MATERIALS AND METHODS

Exfoliative cytology was performed in 10 patients with squamous cell carcinomas of the upper aerodigestive tract without clinical and ultrasonographic evidence of cervical metastases, and in 20 patients with metastases.

In order to improve the yield of cells, swabbing of the tumor was performed by means of a small foam rubber sponge held in a biopsy forceps. Routine cytology, quantitative DNA analysis, and cytomorphologic evaluation were performed. Biopsy specimens for histologic evaluation were taken simultaneously.

In the 20 patients with overt cervical metastases, the metastatic LNs were submitted to biopsy via fine-needle aspiration, or samples from LNs were obtained during the neck dissection.

Histologic and Cytologic Evaluation. Histologic specimens were embedded and stained with hematoxylin and eosin; cytologic specimens were stained with Papanicolaou reagent[21] and with Schiff's reagent according to the method of Feulgen.[22]

For cytomorphologic evaluation and quantitative DNA analysis, an automated video-based image analysis system (DNA-Miamed, Leica Cie, Wetzlar, Germany) was used as previously described by these

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TABLE 1. TUMOR SITES OF PATIENTS WITH N0 (n = 10) AND N+ (n = 20) SQUAMOUS CELL CARCINOMAS OF HEAD AND NECK

Tumor Site	*N0 Tumors*	*N+ Tumors*
Floor of mouth	1	0
Oropharynx	5	12
Hypopharynx	1	5
Larynx	3	3
Total	10	20

authors.[23]

Three hundred tumor cells were measured in each specimen at random after identification on the video screen. For calibration of the diploid DNA content, at least 35 lymphocytes or granulocytes in the same specimen were measured to determine the 2c peak.

The following indices were determined from the single cell DNA:

1. The 2c deviation index (2c DI), defined as the mean square deviation from the diploid value. Values of the 2c DI that exceeded 2.4 were considered pathologic and suspicious for malignancy.[24,25]
2. The 5c exceeding rate (5c ER), defined as the percentage of aneuploid cells with a DNA content of more than 5c.
3. The DNA malignancy grade (DNA MG).
4. The stem line analysis, which defines the DNA content of the predominant cell line in the specimen.
5. The mean DNA content of all cells.

In addition, the following cytomorphologic data were evaluated:

1. The cytomorphologic "form factor," which represents the deviation of the form of a cell nucleus from normal circle form.
2. The mean nuclear area (in square microns), which was computed from the nuclear areas of the single cells.
3. The mean nuclear perimeter (in microns) of the cancer cells derived from the single cell values.

Statistical analysis was performed by using the Mann-Whitney test to compare N0 and lymph node–positive (N+) tumors. The Wilcoxon signed rank test was performed to compare N+ and LN metastases.

TABLE 2. TN CLASSIFICATION OF PATIENTS EXAMINED (UICC 1987)

	N0	*N1*	*N2*	*N3*
T2	3	2	4	0
T3	5	3	5	1
T4	2	1	3	1
Total	10	6	12	2

TABLE 3. RESULTS OF CYTOMORPHOLOGIC EVALUATION

	Form Factor	*Mean Nuclear Perimeter (μm)*	*Mean Nuclear Area (μm^2)*
N0 tumors	1.63 ± 0.09	37.56 ± 2.58	71.28 ± 7.86
N+ tumors	1.98 ± 0.47	43.64 ± 7.46	89.39 ± 25.87
Lymph node metastases	1.88 ± 0.38	46.17 ± 7.58	95.51 ± 24.92

Data are mean ± 1 SD.

RESULTS

We evaluated 10 tumors from patients without clinical metastases (9 male, 1 female, average age 58.4 years) and 20 patients with metastases (17 male, 3 female, average age 53.8 years; Tables 1 and 2). Out of these, 6 tumors were staged as N1, and 14 patients had advanced LN metastases (N2 and N3).

The level of differentiation, using the morphologic criteria of Broders,[26] revealed for the N0 tumors 1 well-differentiated, 7 moderately well-differentiated, and 2 poorly differentiated cancers. The tumors with metastases were well differentiated in 2 cases, moderately well differentiated in 14, and poorly differentiated in 4. This parameter was therefore thought to be of no prognostic value.

Routine cytology revealed malignant criteria in all smears, ie, hyperchromicity, polymorphism, shift of nuclear-cytoplasmic relationship, enlargement of the nuclei with disaggregated chromatin and vacuoles, and occasional karyolysis or pyknosis. There were no differences in the cytologic findings between patients with N0 and N+ tumors or between the primary cancers and the LN metastases.

In a clinical follow-up of the 10 patients without clinical or ultrasonographic evidence of LN metastases, 1 of them developed metastatic disease 3 months after the operation on the primary tumor. Nine patients were without clinical or ultrasonographic evidence of metastases at an average follow-up of 16.8 months (range 11 to 21 months).

Cytomorphologic Evaluation. In N0 tumors the form factor of tumor cells ranged from 1.57 to 1.85,

TABLE 4. RESULTS OF DNA ANALYSIS

	Mean DNA	*2c DI*	*5c ER*
N0 tumors	3.22 ± 0.38	4.18 ± 1.46	9.19 ± 2.87
N+ tumors	3.80 ± 0.30	7.69 ± 4.79	13.54 ± 5.17
Lymph node metastases	4.10 ± 0.59	9.89 ± 5.77	17.98 ± 8.33

Data are mean ± 1 SD.

DI — deviation index, ER — exceeding rate.

TABLE 5. RESULTS OF DNA MALIGNANCY GRADE AND STEM LINE ANALYSIS

	DNA Malignancy Grade	*Stem Line Analysis*
N0 tumors	1.23 ± 0.23	2.27 ± 0.24
N+ tumors	1.57 ± 0.32	2.79 ± 0.72
Lymph node metastases	1.72 ± 0.38	3.23 ± 0.77

Data are mean ± 1 SD.

with an average of 1.63 ± 0.09. The mean area of nuclei was 71.28 ± 7.86 μm^2 (range 62.28 to 84.60 μm^2). The mean nuclei perimeter ranged from 34.35 to 40.99 μm (average 37.56 ± 2.58 μm).

In N+ tumors the mean form factor ranged from 1.26 to 2.46, with an average of 1.98 ± 0.47. The mean nuclear areas were widespread and ranged between 41.18 and 151.55 μm^2 (average 89.39 ± 25.87 μm^2). The mean nuclear perimeter varied between 24.31 and 53.20 μm (average 43.64 ± 7.46 μm).

Analysis of the LN metastases revealed a mean form factor between 1.35 and 2.89 (average 1.88 ± 0.38). The mean nuclear size was highest in this group, with an average value of 95.51 ± 24.92 μm^2 (range 49.66 to 154.51 μm^2). The mean nuclear perimeter varied between 31.34 and 58.28 μm (average 46.17 ± 7.58 μm).

Statistically, there appeared to be a significant association of mean nuclear area (p = .006) and mean nuclear perimeter (p = .0026) when comparing N0 with N+ tumors. Tumor cells in N+ tumors had an increased area of cell nuclei, and mean nuclear area was highest in the LN metastases. No significant difference could be detected when the form factors (p = .94) and mean nuclear perimeters (p = .11) of N+ tumors and LN metastases were compared (Table 3).

Analysis of the cytomorphologic data of patients with N1 tumors and those with advanced LN metastases revealed no statistically significant differences. Likewise, no statistically significant differences were found in the cytomorphologic data when tumors were grouped by T classification.

Quantitative DNA Analysis. The mean DNA content was highest in cells obtained from the LN metastases and lowest in N0 tumors. The differences between mean DNA content in N0 tumors and N+ tumors (p = .0013) and between N+ tumors and LN metastases (p = .038) were significant. Also, significant differences were found between the 2c DI in N0 tumors and N+ tumors (p = .0029) and between N+ tumors and LN metastases (p = .041). The N0 cells had a smaller number of aneuploid cells with a DNA content exceeding 5c compared to N+ cells (p = .03), as did N+ tumor cells compared with the cells from the corresponding LN metastases. The latter difference was not, however, statistically significant (p = .072; Table 4).

The DNA MG in N+ tumors was higher than in N0 tumors (p = .0019). It was also higher in 14 of the 20 LN metastases when compared to the values of the primary tumors; however, this difference was not statistically significant (p = .135). Stem line analysis showed nondiploid stem lines that increased 2.5c in 4 of 10 N0 cases, 11 of 20 N+ tumors, and in all of the LN metastases. The differences between N0 cases and N+ primaries (p = .042) and between N+ primaries and the corresponding LN metastases (p = .024) were significant. The results of the DNA MG and the

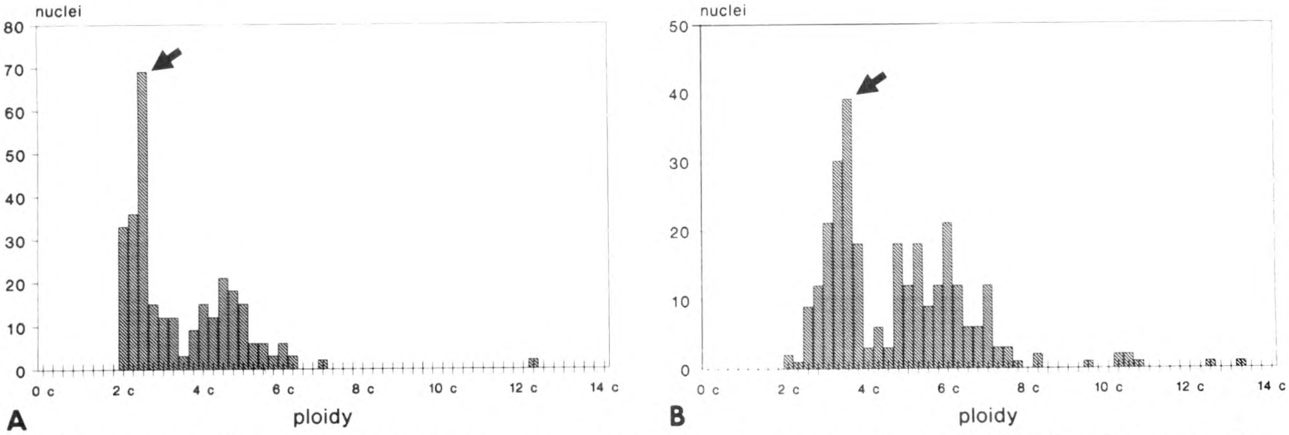

Fig 1. Results of calibrated DNA analysis of primary tumor and corresponding lymph node metastasis from 51-year-old man with carcinoma of oropharynx, T4N2bM0, judged on histology to be moderately well-differentiated squamous cell carcinoma. Arrow — stem line. A) Primary tumor. Mean DNA content was 3.44, 2c deviation index 8.45, 5c exceeding rate 13, DNA malignancy grade 1.71, and stem line value 2.3 c. B) Corresponding lymph node metastasis. Mean DNA content was 5.17, 2c deviation index 18.66, 5c exceeding rate 40, DNA malignancy grade 2.27, and stem line value 3.22 c. Note increased values for mean DNA content, 2c deviation index, 5c exceeding rate, and DNA malignancy grade and shift of stem line toward aneuploidy.

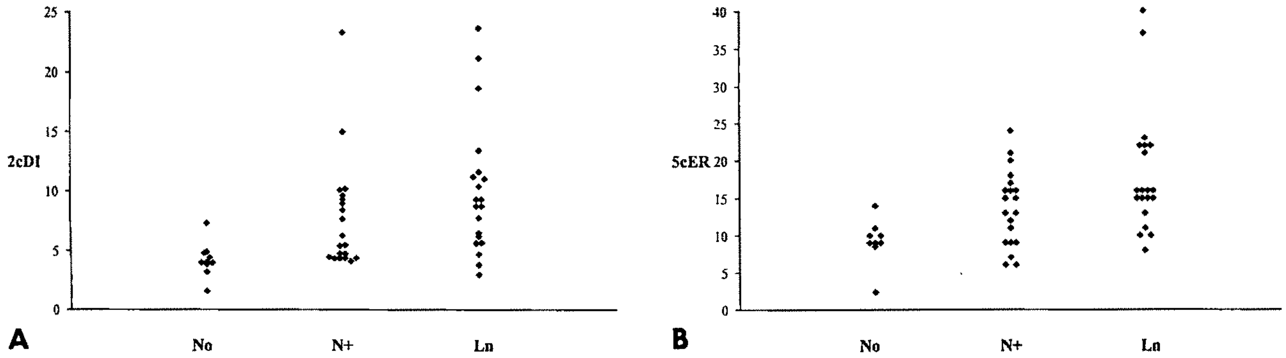

Fig 2. Scatter diagram of A) 2c deviation index (DI) and B) 5c exceeding rate (ER) in N0 tumors, N+ tumors, and lymph node (Ln) metastases.

stem line analysis are summarized in Table 5.

The highest values for the mean DNA content, 2c DI, 5c ER, and stem line were noted in tumors that were histologically described as poorly differentiated squamous cell carcinomas.

An example of a DNA analysis conducted on a primary tumor with the corresponding LN metastasis is detailed in Fig 1. Figure 2 shows a scatter diagram of the results of 2c DI and 5c ER in N0 tumors, N+ tumors, and LN metastases.

The population of N+ tumors was then grouped in patients with N1 lesions and those with advanced nodal metastases. Tumor cells in tumors with advanced LN metastases revealed statistically significant higher values for 2c DI and DNA MG (Table 6). No statistically significant differences were found when the tumors were grouped by T classification.

DISCUSSION

The histologic differentiation between malignant and benign lesions based on light microscopy evaluation is acceptably accurate. Likewise, cytologic evaluation of smears of mucosal lesions in the upper aerodigestive tract have been proven to be suitable for detection of malignancy.[27-29] However, Gaafar et al[30] noted a 6.7% false-negative rate in cytologic smears taken from laryngeal tumors when compared to the histologic diagnosis. The disadvantage of both cytologic and histologic evaluation is that the diagnosis and grading are based on morphologic criteria, are subject to differences in individual interpretation, and have proved not to be reliable as prognostic indicators. In addition, malignancy grading from cytologic smears based on cell differentiation is not possible.[31]

TABLE 6. RESULTS OF QUANTITATIVE DNA MEASUREMENTS

	*N1 Tumors (n = 6)**	*N2 and N3 Tumors (n = 14)**	*p*
Mean DNA	3.36 ± 0.22	3.95 ± 0.55	.022
2c deviation index	4.17 ± 0.52	8.88 ± 2.91	.032
5c exceeding rate	12.23 ± 3.55	13.93 ± 5.52	.522
DNA malignancy grade	1.27 ± 0.09	1.67 ± 0.32	.007
Stem line	2.47 ± 0.43	2.93 ± 0.65	.178

*Data are mean ± 1 SD.

For squamous cell carcinomas, the depth and mode of invasion have to date been the most precise prognostic indicators, particularly regarding the probability of LN metastases.[2-4] Cytology findings to date have never been very conclusive as a prognostic indicator.

Quantitative DNA measurements, on the other hand, have demonstrated reliability as prognostic indicators in head and neck tumors, and close correlations among ploidy, DNA index, and survival time have been mentioned.[32-37] These authors suggested that aneuploidy and an increased DNA index are important prognostic factors. Crissman and Zarbo[38] recognized aneuploid cells in squamous intraepithelial neoplasia of the larynx by DNA measurements, which correlated with the development of invasive cancer.

In this study the results of the DNA measurements showed an increased mean DNA content, an increased value for 2c DI, an increased percentage of aneuploid tumor cells, and, most frequently, an aneuploid stem line in tumors with metastases as compared to tumors without clinical or ultrasonographic evidence of metastases. The highest measurements were obtained from the metastases themselves. These data suggest that the appearance of aneuploid tumor cell clones is related to the probability of metastases, and that highly aneuploid stem lines in primary tumors make the appearance of metastases very likely.

We were also able to demonstrate that in this group of patients studied in our institution the DNA data obtained from primaries and LN metastases showed considerable differences. Likewise, the DNA data of the LN metastases showed differences. Tumor cells in advanced LN metastases revealed the highest values for 2c DI and DNA MG. It is a matter of speculation whether these differences are related to the variant responsiveness of primaries and LN metastases to chemotherapy and radiotherapy.

Our results are in accordance with other studies of DNA analysis in head and neck tumors.[39,40] Tytor et al[20] found increased values for DNA content and aneuploid tumor cells in N+ tumors of the oral cavity when compared to N0 primaries. In most of the N+ cases, the stem lines were aneuploid and there was a close relationship between progression of the metastases and the aneuploidy of the primary tumors.[20]

In conclusion, quantitative DNA analysis of primary squamous cell carcinomas of the head and neck may have a predictive value for the development of LN metastases. These findings may have therapeutic implications, eg, determine which patients should undergo treatment of the neck or determine who may benefit from additional, more aggressive treatment such as chemotherapy or irradiation.

The discrepancies between the DNA data in primary tumors and LN metastases may be an indicator as to why primary tumors appear to be more sensitive to chemotherapy than their LN metastases.

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NEURORADIOLOGY AND HEAD AND NECK RADIOLOGY

The 12th Annual Comprehensive Update: Neuroradiology and Head and Neck Radiology (with emphasis on MR and CT of the brain, head, neck and spine) will be held February 25-28, 1993, at Buena Vista Palace, Walt Disney World® Village, Lake Buena Vista, Florida. For further information, contact Ms Kathi Goodwin, Department of Radiology, SUNY Health Science Center at Syracuse, 750 East Adams St, Syracuse, NY 13210; (315) 464-7408, FAX (315) 464-7458.

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MEASUREMENT OF MUCOSAL WAVE PROPAGATION AND VERTICAL PHASE DIFFERENCE IN VOCAL FOLD VIBRATION

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Examination of the surface wave properties of the vocal fold mucosa is becoming an important part of assessment of vocal function. A key wave property is propagation velocity, which determines the phase delay between the upper and lower margins of the vocal folds. Excised canine larynges were used to measure this phase delay, and therewith propagation velocity. The motion of two flesh points was tracked stroboscopically. Differential displacements between the flesh points were matched to displacements of a model. A least-squared fit of the data to the model provided the numeric values of propagation velocity, which varied from 0.5 m/s to about 2.0 m/s, depending on fundamental frequency. The corresponding phase delay along the medial surface of the vocal folds varied from about 60°/mm to 30°/mm.

KEY WORDS — glottis, mucosal wave, vocal fold vibration, voice.

INTRODUCTION

It is now well established that self-oscillation of the vocal folds is facilitated by a phase delay in movement of the upper margin of the folds with respect to the lower margin. Ishizaka and Matsudaira[1] demonstrated this by deriving analytic expressions for conditions of oscillation with a two-mass model of the vocal folds. Subsequently, Titze[2] confirmed the derivation by modeling a surface wave that propagated vertically along the medial surface of the vocal fold mucosa. A finite propagation velocity of the mucosal wave supplies the phase delay, and thus the two approaches have merged conceptually into one theory of vocal fold vibration. The theory is based on small-amplitude assumptions, with flow-induced vibration being sustained around a small glottal opening.

More recently, the clinical importance of the mucosal wave has been described by Bless et al.[3] Patients with vocal fold lesions and scarred tissue in the mucosa show a reduced amplitude of the mucosal wave. Furthermore, the phonation threshold pressure (the minimum lung pressure required to establish phonation at a given pitch) has been shown to be related to the mucosal wave velocity,[4,5] suggesting that "ease of phonation" is facilitated by a greater phase delay.

Given these theoretic and clinical observations, it seems logical to conduct an investigation to measure the phase delay and the associated mucosal wave velocity. In particular, it is important to know how these quantities vary with the fundamental frequency (F_0). This would then allow better interpretation of phonation threshold pressure, which is known to vary nonlinearly with the F_0.[4] One criterion for successful phonosurgery might be the lowering of phonation threshold pressure,[6] which expands the range of intensity that can be achieved in phonation.

Relations Between Mucosal Wave Velocity and Vertical Phase Delay. Hiroto[7] was one of the first to point out that the maximum glottal width, as seen from above, depends on time-delayed movements between the upper and lower margins of the vocal folds. More recently, Hanson et al[8] and Berke et al[9] confirmed this finding with glottographic measurements conducted on human subjects and canine in vivo preparations, respectively. It is understood that glottal width is a time- and space-dependent function that varies in both the anterior-posterior direction and the inferior-superior direction. It can be identified at two vertically separated flesh points on the medial surface of the folds, as shown in Fig 1A. At these points, the time variation of the glottis is modeled as

$$g_1(t) = \mathrm{Max}[0,\ g_{01} + 2a_1\sin(2\pi F_0 t)] \tag{1}$$

and

$$g_2(t) = \mathrm{Max}[0,\ g_{02} + 2a_2\sin(2\pi F_0 t - \phi)] \tag{2}$$

where the subscript 1 refers to the lower flesh point and subscript 2 to the upper flesh point. The quantities g_{01} and g_{02} are prephonatory glottal widths, a_1 and

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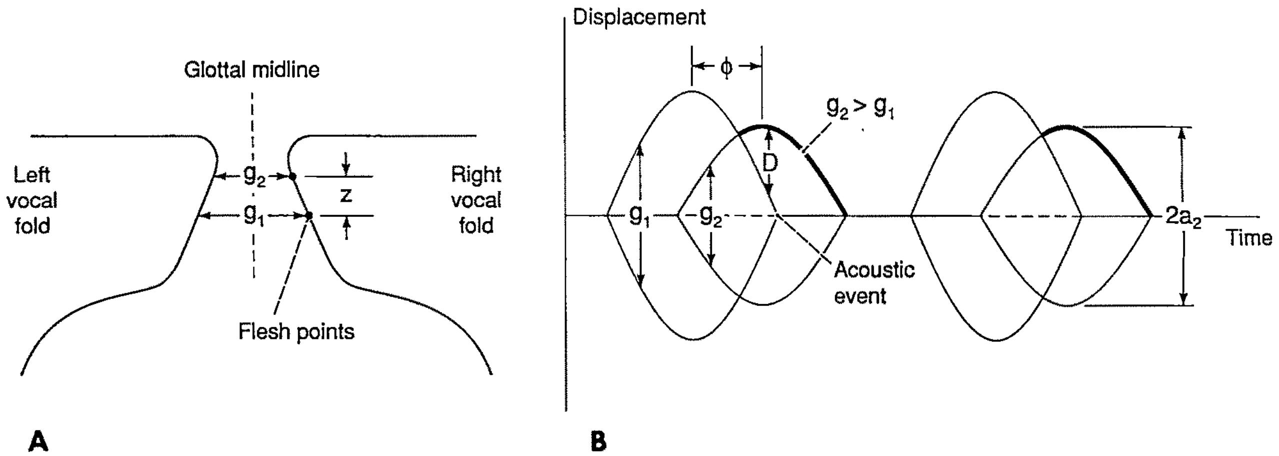

Fig 1. Illustration of movement of flesh points on upper and lower margins of vocal folds. A) Frontal section through vocal folds. B) Displacement versus time for glottal width. Heavy lines illustrate portions of cycle over which measurements can be made. See text for explanation of other symbols.

a_2 are the amplitudes of (assumed) sinusoidal flesh point motions in the medial-lateral direction, and ϕ is the vertical phase delay. An assumption of symmetry between left and right vocal fold movements is implied in the equations, as indicated by the factor of 2 in front of the amplitudes of vibration. Time variations $g_1(t)$ and $g_2(t)$ are shown in Fig 1B for two cycles of vibration. (Some features of this Figure will be explained later.)

It should be kept in mind that the specific glottal widths $g_1(t)$ and $g_2(t)$ are defined only for one common anterior-posterior position. A more complete model of static and dynamic glottal shapes, involving variations along the length of the vocal folds, has been proposed,[10] but this additional complexity is not needed for the present study.

Assuming a surface wave to be propagating vertically upward in the mucosa with a velocity c, the time delay between the two flesh points separated by a vertical distance z is

$$\tau = z/c \tag{3}$$

and the corresponding phase delay is this time delay normalized to the fundamental period of vibration T,

$$\phi = 2\pi\tau/T = 2\pi F_0\tau = 2\pi F_0 z/c \tag{4}$$

If we know the flesh point separation z (Fig 1A) and the fundamental frequency F_0, a measurement of the phase angle ϕ will reveal the propagation velocity c of the mucosal wave. The experimental procedure for obtaining these measures will now be described.

MATERIALS AND METHODS

Excised canine larynges were harvested from mongrel dogs (body weights 20 to 23 kg). The dogs had been killed painlessly in a cardiovascular research laboratory. Only grossly normal larynges were used in this study. From approximately 10 larynges that were harvested, 5 were judged normal and kept for experimentation. Rejection was based on insufficient size, evidence of disease, or obvious asymmetries across the midline.

Techniques for dissecting, mounting, and controlling the glottal configuration of the larynx are described elsewhere.[11] Pressurized air with controlled temperature and humidity was used to induce self-oscillation of the vocal folds, as described by Baer,[12] Durham et al,[11] Cooper,[13] and Yanagi et al.[14]

A custom-designed triggering circuit was used to phase-lock the flashes of a stroboscopic light source (Pioneer DS 330-ST) to specific events in the period of vibration. The stroboscopic image of the top view of the folds, which filled the entire screen of a 19-in video monitor, could thus be frozen to make measurements (in screen coordinates) of glottal dimensions at any given phase in the cycle. Magnification was 10:1, based on an object-lens distance of 45 cm (Sony DXC-102 video camera with a 90-mm microlens).

Prior to mounting the larynx, we placed two small suture marks at the middle of the membranous vocal folds using 9-0 nylon stitches. One mark was at the upper margin and the other approximately 2 mm lower. The vertical distance between these two marks was measured with a micrometer (0.1-mm accuracy) and constituted the distance z in Fig 1A and in equations 3 and 4.

Different subglottal pressures and different elongations of the vocal folds were used to obtain different F_0 values. The two audio channels of a high-fidelity video cassette recorder were used to record two time-locked acoustic signals alongside the stroboscopic video image. One acoustic signal was the audio signal from a microphone (Realistic 33-1056A)

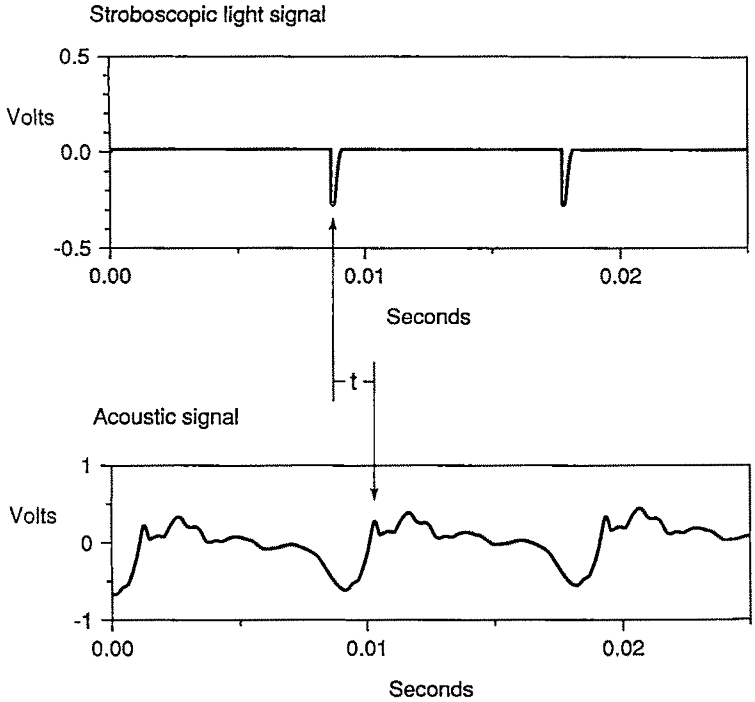

Fig 2. Display of simultaneously recorded strobe flash signal (upper trace) and microphone signal (lower trace). Time interval (t) is relative time between two identifiable events in cycle.

placed 5 cm from the vocal folds, and the second signal was a timing signal obtained from a light sensor that converted the stroboscopic flashes into electrical signals. In this manner, video events could be correlated with a constant acoustic event in the vibratory cycle. Figure 2 shows an example of the two time-locked audio signals. The interval t defines the time between the strobe flash (top trace) and the initial burst of acoustic pressure and glottal closure (bottom trace). The burst of acoustic pressure was taken to be the constant reference event, also shown in Fig 1B. Phase differences between flesh point movements could then be determined relative to this reference event and relative to each other.

Data Reduction. During video playback, the lateral displacement D between the two marks was measured for each of a number of phases. According to equations 1 and 2, this displacement is

$$(5)\quad D = g_2 - g_1 = (g_{02} - g_{01}) + 2a_2\sin(2\pi F_0 t - \phi) - 2a_1\sin(2\pi F_0 t)$$

The quantity D and the phase angle ϕ are shown in Fig 1B. Only those phases in which g_2 was greater than g_1 could be used for measurement (thick portion of g_2), because in all other cases the upper mark

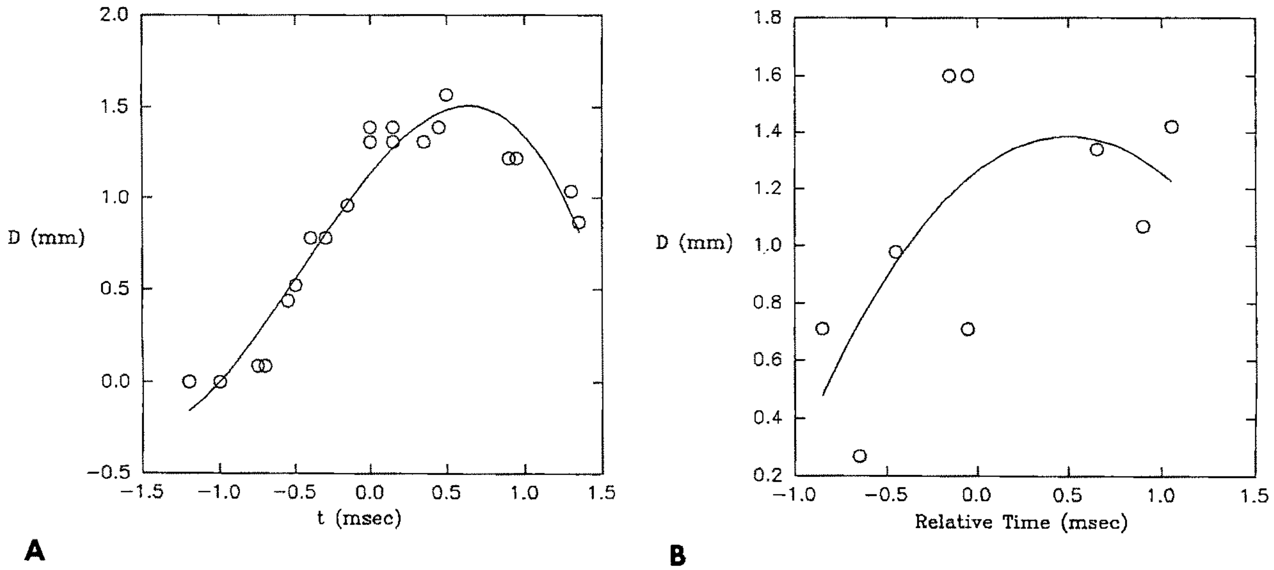

Fig 3. Distance (D) between two marks on vocal folds as function of relative time (t) for A) best case and B) worst case. Solid curve is model given by equation 5.

MUCOSAL WAVE VELOCITY AND CORRESPONDING PHASE DELAY

Larynx No.	F_0 *(Hz)*	*Velocity c (m/s)*	*Phase delay* ϕ/z *(°/mm)*
1	95	0.587	58.3
	107	0.877	43.9
	114	0.673	61.0
	119	0.704	60.9
	123	0.858	51.6
	131	0.778	60.6
	134	0.817	59.1
	140	0.847	59.5
	157	0.946	59.8
	173	1.466	42.5
2	129	0.866	53.6
	136	0.934	52.4
	154	1.047	53.0
	163	1.124	52.2
	178	1.139	56.3
3	126	0.859	52.8
	141	0.940	54.0
	158	1.063	53.5
	163	1.086	54.1
	181	2.081	31.1
4	127	1.012	45.2
	134	1.147	42.1
	139	1.254	39.9
	148	2.177	24.5
5	137	0.951	51.8
	148	1.469	36.3
	152	1.764	31.0
	155	2.001	27.0

F_0 — fundamental frequency, ϕ — phase angle, z — flesh point separation.

shadowed the lower mark. Given that the glottis is closed over about half of the period, and given that over about one quarter of the period the bottom mark is shadowed, the average measurement period was only about one quarter of a cycle (duration of thick line).

The acoustic signals from audio playback were displayed on a digital oscilloscope (Data 6000). The stroboscopic reference time t was measured along with each value of D. More than 10 sets of D versus t data could be obtained for each pitch of a given larynx. To determine additional unknown factors in equation 5, the maximal glottal width at the upper flesh point ($2a_2$, shown in Fig 1B) was also measured separately on the video screen. Finally, the quantity $g_{02} - g_{01}$ was determined to be negligible, because the marks were only about 1.5 to 2.0 mm apart and the prephonatory glottis was nearly rectangular. This left only a_1 and ϕ as unknowns.

Using nonlinear regression curve fitting (Marquardt-Levenberg algorithm in Sigmaplot 4.0 program), we fitted the data to equation 5, where a_1 and ϕ were parameters to be optimized under program control. The criterion for optimization was the combined least-squared difference between the measured and calculated Ds for the 10 or more data points.

Examples of best-case and worst-case matches between the measurement and the model of D as a function of t are given in Fig 3. In the best case (Fig 3A), a sinusoidal difference function for D can readily be seen, both for the data and the model. (The difference between two sinusoids of different phase and different amplitude is also a sinusoid.) In the worst case (Fig 3B), the sinusoidal nature of the data was obscured, but the model was nevertheless deemed appropriate. Note that the curves follow the pattern one would predict from Fig 1B. The difference function D is at a maximum near $t = 0$, the acoustic reference event, and diminishes in both directions on the thick line.

RESULTS

The Table shows data of mucosal wave velocity c and phase delay per millimeter (ϕ/z) from five excised canine larynges. At least four different F_0 values were obtained on each larynx. The columns of data are related by the equation

$$\phi/z = 360F_0/c \qquad (6)$$

which is equation 4 expressed in degrees rather than radians. Note that the velocities range from about 0.6 m/s to about 2.2 m/s, with higher values consistently corresponding to higher F_0 values. The phase delay per millimeter decreases with F_0.

Figure 4 shows the data of the Table graphically. Except for larynx 5, which shows an unusually steep rise of c with F_0 (and a correspondingly steep fall of ϕ/z), the results suggest that wave velocity in the mucosa may increase linearly with F_0. A threefold increase in c, from 0.5 m/s to 1.5 m/s, is observed over the 100- to 180-Hz range. If larynx 5 were excluded., the increase would still be more than twofold. Phase delay may decrease linearly, but at a relatively moderate rate of decline (less than 30% over the 100- to 180-Hz range, and less if larynx 5 is excluded).

The rate of decline of ϕ/z with F_0 may actually be overestimated. As the vocal folds are elongated, they usually shrink in thickness to preserve overall tissue volume. A 30% elongation, for example, would decrease the separation z of the marks by 30%. This would then keep ϕ/z nearly constant with F_0, and c would increase in proportion to F_0. Further investi-

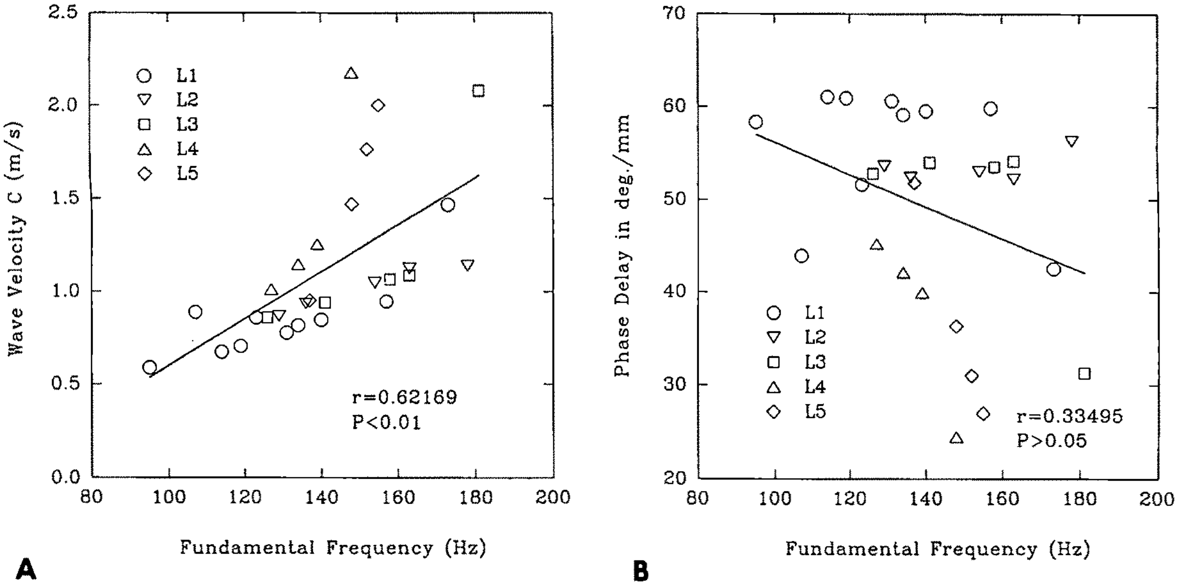

Fig 4. Data from Table, shown graphically. A) Wave velocity c versus fundamental frequency for all five larynges. B) Phase delay in degrees per millimeter as function of fundamental frequency for all five larynges.

gation of this effect is needed.

DISCUSSION AND CONCLUSIONS

In experiments conducted on excised canine larynges, Baer summarized his findings with respect to mucosal wave velocity as follows.

> The derived propagation velocities are on the order of 1.0 m/s. This value may be compared with the value of 1.6 m/s reported by van [*sic*] Gierke et al (1952) on human skin and in such places as the thigh or forearm. As discussed earlier, however, wave velocity on the more lateral parts of the superior surface drops to 0.3 to 0.5 ms, perhaps because the membranes are more slack or because the effective thickness is greater.[12(p190)]

The article by Von Gierke et al[15] is also worthy of note. It is often quoted as an authoritative reference on human skin vibration.

It is clear from Fig 4A that Baer's value 1.0 m/s is in the center of the range measured here. Our findings, therefore, agree with his. Slower wave velocities (0.1 to 0.5 m/s) were measured by Hirano et al[16] on the superior surface. These findings do not conflict with our results in light of Baer's comment that mucosal waves slow down considerably as they propagate laterally. It is important, therefore, to specify where the wave velocity is measured. Hirano et al did this carefully in their study.

The importance of quantifying the mucosal wave velocity on the medial surface stems from the fact that phonation threshold pressure, the minimum pressure required to establish phonation, depends critically on this propagation velocity.[2] Thus, by knowing how c varies with F_0 on this surface, a better understanding of phonation threshold pressure may emerge in the future. Direct or indirect estimates of this pressure may then lead to better tools for assessment of normal and abnormal vocal function.

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INTERNATIONAL HEARING AID CONFERENCE
GENERAL ANNOUNCEMENT AND CALL FOR PAPERS

An International Hearing Aid Conference will be held June 25-27, 1993, at The University of Iowa, Iowa City. For information on registration and accommodation, contact the Conference Center, University of Iowa, Memorial Union, Iowa City, IA 52242; (319) 335-3231, Fax (319) 335-3407. For information on Call for Papers, contact Regina Tisor (319) 356-2471, fax (319) 356-4547. We have applied for Continuing Education Units from ASHA and HAIC.

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FIRST CONGRESS OF EUROPEAN SKULL BASE SOCIETY

The European Skull Base Society will hold its first congress September 26-30, 1993, in Riva del Garda (Trento), Italy. For further information contact Carmelo Zappone, MD, Congress Secretary, Division of ORL, Ospedali Riuniti, Largo Barozzi 1, 24100 Bergamo, Italy; telephone 35-269578; fax 35-269460.

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ACCUMULATION OF POTASSIUM IN SCALA VESTIBULI PERILYMPH OF THE MAMMALIAN COCHLEA

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Movements of potassium (K^+) were monitored during perfusion of either the scala tympani (ST) or the scala vestibuli (SV) of the guinea pig cochlea with a solution containing 15 mmol/L K^+. A highly asymmetric clearance of K^+ was observed, with K^+ rapidly being taken up from the ST and allowed to accumulate in the SV. Under some conditions the SV K^+ concentration could exceed that in the perfused ST. These observations are believed to result from the distortion of passive K^+ diffusion by the high circulating current of K^+ that is part of the transduction process. Calculations are presented to demonstrate that circulating fluxes are of sufficient magnitude to generate the results observed. The high rate of circulating K^+ current is probably also responsible for the difference in physiologic K^+ concentration between the ST and SV, in which the ST perilymph K^+ concentration is typically found to be half that of the SV. A clearance of K^+ from the ST and its eventual accumulation in the SV could play a role in how the ear responds to abnormal ion concentrations, such as may occur in Meniere's disease. It is proposed that an accumulation of K^+ in the SV would result in vestibular dysfunction that might contribute to the vestibular symptoms of the disease.

KEY WORDS — cochlear fluids, Meniere's disease, perilymph.

INTRODUCTION

Potassium (K^+) is the major cation in cochlear endolymph[1] and plays a major role in the cochlear transduction process. Although ionic replacement studies have shown that the transduction channels of vestibular hair cells can be characterized as nonspecific cation channels,[2] there is no doubt that in the normal state most of the circulating transduction current is carried by K^+, since it is the predominant cation present on either side of the apical membrane of the hair cell. This is supported by studies in which the intracellular microphonic recorded from inner hair cells of the guinea pig cochlea reversed phase when the intracellular potential was close to the endocochlear potential, ie, when the driving gradient for K^+ across the apical hair cell membrane was near zero.[3] Potassium movements are thus believed to play a prominent role in cochlear transduction.

Cochlear perilymph shows systematic inhomogeneities with respect to K^+. Many studies have reported that the K^+ concentration in scala vestibuli (SV) perilymph is approximately twofold higher than that in the scala tympani (ST).[4] However, in most published studies, precautions have not been taken to prevent the nonphysiologic influx of cerebrospinal fluid (CSF) into the ST on perforation of the cochlea.[5,6] Since CSF has a lower K^+ content than perilymph (typically less than 3 mmol/L), it is possible that lower K^+ concentrations for ST perilymph may really reflect the greater difficulty of obtaining uncontaminated perilymph from the ST.[7] The possibility of a twofold difference in resting K^+ levels is difficult to reconcile with the high rate of passive communication across the spiral ligament shown by Salt et al.[8] That study used trimethylphenylammonium (TMPA) to explore the passive communication of small solutes between the ST and SV. The TMPA was perfused through one scala while the time course of entry was monitored in the opposite, nonperfused perilymphatic scala. The results showed that in the third turn the rate of cross-communication was rapid, with a rate constant from ST to SV of 0.049 minute^{-1} (half-time, 14.2 minutes) and from SV to ST of 0.03 minute^{-1} (half-time, 22.4 minutes).

In the present study, we have measured the steady-state, resting concentrations of K^+ in the ST and SV under experimental conditions specifically designed to avoid artifacts associated with perforation of the otic capsule. We have further investigated the effect of inducing changes in the K^+ concentration of one scala by monitoring K^+ concentrations simultaneously in both perilymphatic scalae. The goal of the study was to verify the existence of a concentration difference for K^+ between the ST and SV and to clarify how the difference arises.

METHODS

Healthy pigmented guinea pigs weighing 200 to 400 g were anesthetized with sodium pentobarbital (33 mg/kg). Surgical anesthesia was maintained

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From the Department of Otolaryngology, Washington University Medical School, St Louis, Missouri. This work was supported by National Institutes of Health grants DC00384 and DC01368. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Washington University.

REPRINTS — Alec N. Salt, PhD, Dept of Otolaryngology, Washington University Medical School, 517 S Euclid Ave, St Louis, MO 63110.

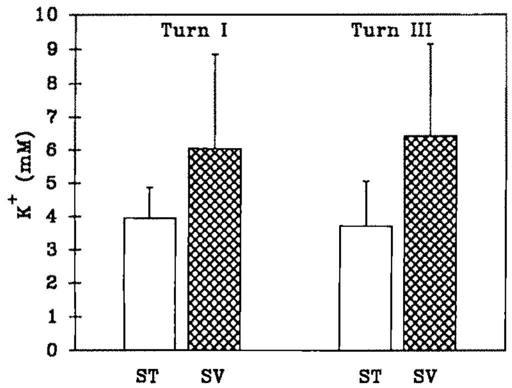

Fig 1. Summary of physiologic potassium (K^+) concentrations in scala tympani (ST) and scala vestibuli (SV) of guinea pig cochlea. Measurements were made by K^+-selective electrodes via procedures designed to minimize perilymph loss due to cochlear perforation. Values are average of four measurements in first turn and three measurements in third turn. Bars — SD.

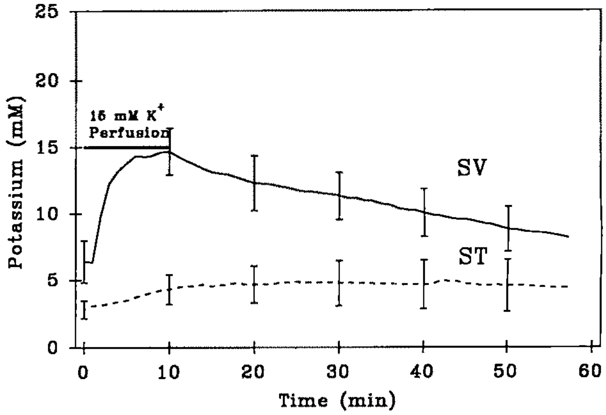

Fig 2. Potassium concentration changes measured simultaneously in scala tympani (ST) and scala vestibuli (SV) of second turn during and after perfusion of SV with 15 mmol/L potassium solution. Potassium concentration in ST is only slightly elevated. Curves are average of three experiments with points at 1-minute intervals. Bars — SD (shown only at 10-minute intervals for simplicity).

throughout the experiment by giving supplementary doses via an intravenous cannula. The left cochlea was exposed by a ventrolateral approach. During data collection, the animals were immobilized with gallium triethiodide (2 to 3 mg/kg) and were artificially ventilated.

The K^+ content of perilymph was measured by ion-selective electrodes that were inserted into the perilymph through small (30 to 50 μm in diameter) fenestras made in the bone. In perfusion experiments, Permabond (Englewood, NJ) 101 adhesive was used to establish a fluid-tight seal between the electrode and the cochlea as described in detail by Ohyama et al.[9] Potassium-selective electrodes were made from double-barreled glass pipettes with their tips broken to 2 μm. One barrel of the pipette was silanized by exposure to dimethyldichlorosilane vapor. The silanized (ion) barrel was filled with 500 mmol/L potassium chloride (KCl), and the other (reference) barrel was filled with 500 mmol/L sodium chloride (NaCl). A small column of Fluka 60398 ion-exchanger (Fluka Chemical, Ronkonkoma, NY) was drawn into the ion barrel by suction. Electrical connections were made via silver–silver chloride wires to a multichannel, custom-made electrometer that used AD515L electrometer amplifiers (input impedance greater than 10^{15} Ω). Potentials were read from integrating digital voltmeters (Keithley 197) equipped with IEEE-488 interfaces. When used in endolymph, the electrodes were calibrated in three standards containing 100, 150, and 200 mmol/L KCl. For the perilymph measurements, the electrodes were calibrated in five standards containing from 2 to 40 mmol/L KCl in various backgrounds of NaCl to make a total concentration of 150 mmol/L. In both conditions, the electrode responses were linearly related to the logarithm of the K^+ concentration. During the experiments, the electrode potentials were sampled and stored at 10- to 20-second intervals under the control of a microcomputer. Samples were reduced off-line to 1-minute intervals by linear averaging. Averaged time courses were calculated from the 1-minute interval data. For simplicity, standard deviation bars are shown in the illustrations only at 10-minute intervals.

Perfusion of one scala was achieved by sealing a pipette into the basal turn and making an outlet at the apex. Fluid injection was performed at a rate of 4.5 μL/min with a syringe pump. The perfusate consisted of an artificial perilymph, modified to increase K^+ and decrease sodium (Na^+). It contained 15 mmol/L of KCl, 113.5 (mmol/L) of NaCl, 25 of sodium bicarbonate, 1.2 of magnesium chloride, 0.75 of sodium biphosphate, 1.3 of calcium chloride, and 5 of glucose. The perfusate was bubbled with 95% oxygen and 5% carbon dioxide and checked to be in the pH range of 7.2 to 7.4. During perfusion the experimental chamber was warmed to over 30°C to minimize cooling effects. In perfusion experiments, the effects of CSF influx were minimized by releasing CSF pressure. The dura mater in the region of the foramen magnum was opened, and a short length of polyethylene tubing was inserted to keep the opening patent.

RESULTS

The physiologic levels of K^+ in the SV and ST were measured in experiments in which double-barreled K^+-selective electrodes were inserted into the intact cochlea through very small fenestras. Only those experiments in which ST and SV measurements were made with negligible fluid loss were accepted into

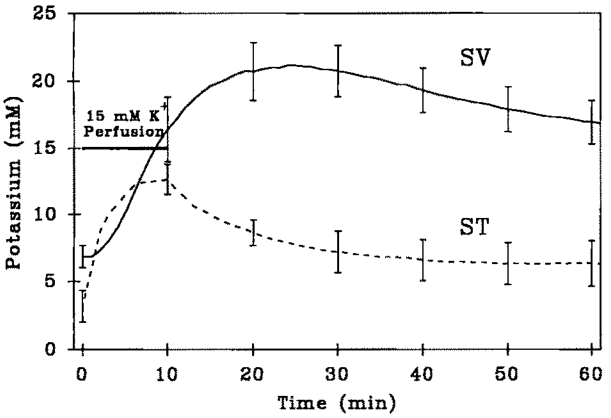

Fig 3. Potassium concentration changes measured simultaneously in scala tympani (ST) and scala vestibuli (SV) during and after perfusion of ST with 15 mmol/L potassium solution. Following perfusion, potassium is rapidly cleared from ST. Potassium concentration in SV increases during perfusion, even though this scala is not perfused. Concentration rapidly exceeds perfused concentration and only slowly recovers toward normal value. Curves are average of four experiments with points at 1-minute intervals. Bars — SD (shown at 10-minute intervals).

the study. As soon as a stable electrode reading was obtained, the electrode was withdrawn and the access fenestra was filled with bone wax to prevent further fluid leakage. Figure 1 summarizes the successful pairs of measurements made in three animals in the third turn and in four animals in the basal turn. In both the basal and third turns, the perilymph K+ level was systematically lower in the ST than it was in the SV. Furthermore, there were no systematic differences in K+ between the first and third turns. These findings confirm that a physiologic difference in K+ concentration exists between the ST and SV throughout the cochlea and under conditions in which perilymph leakage is carefully limited.

Experiments were performed to investigate how the physiologic difference in K+ concentration shown above could exist in the presence of the previously demonstrated passive cross-communication of small ions across the spiral ligament. This was investigated by a method similar to that used in studies previously performed for TMPA.[8] The normal K+ level in one scala was elevated by perfusion with 15 mmol/L K+ solution while the K+ levels were monitored simultaneously in the perfused and nonperfused scalae. The recordings were made in the second cochlear turn. The averaged time courses for SV perfusion with 15 mmol/L K+ solution in three animals are illustrated in Fig 2. Perfusion was for a period of 10 minutes, followed by 50 minutes with the pump off. The amount of cross-communication, inferred from the amount of K+ entering the ST from the SV, appears to be very small. During the period of observation the ST concentration only increased by a mean value of 2 mmol/L. The recovery of the SV after perfusion occurred slowly and in an approximately exponential fashion. The half-time of an exponential curve that fitted the decline was 51 minutes.

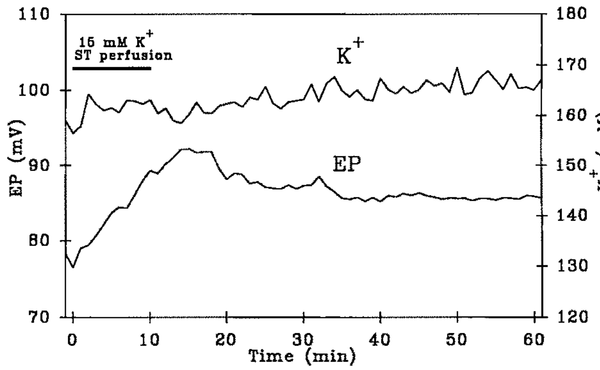

Fig 4. Typical endocochlear potential (EP) and endolymph potassium (K+) changes as result of scala tympani (ST) perfusion with 15 mmol/L K+ solution. Endocochlear potential shows marked increase, but K+ does not systematically change.

Considerably different results were obtained in four oppositely directed experiments, in which the ST was perfused with 15 mmol/L K+ solution, as summarized in Fig 3. The time course in the perfused scala during perfusion was similar to that above, although the recovery toward the normal value after perfusion occurred considerably faster. In this case the half-time of an exponential curve fitted to the averaged recovery time course was 7.2 minutes. This time course is too rapid to be affected by the residual rate of CSF entry, which is estimated to be around 7 nL/min under the experimental conditions used.[6] Since the ST volume between the cochlear aqueduct and the recording site in the second turn is approximately 7 μL, it is certain that local mechanisms predominate. The behavior of the nonperfused scala (SV) was also markedly different for ST perfusion. The SV K+ concentration increased rapidly during perfusion and in all cases exceeded the concentration of the perfused scala within the 10-minute perfusion period. The concentration continued to increase after perfusion ceased, reaching a mean maximum value of 21.2 mmol/L, some 6.5 mmol/L above the highest concentration in the perfused scala. This result clearly cannot be accounted for by passive movement of K+, because the K+ concentration in the SV should never exceed that of the perfused scala. This result suggests the existence of an apparent mechanism of K+ clearance from the ST directed toward the SV.

The possible involvement of the endolymphatic system in this process was investigated by monitoring the endocochlear potential (EP) and endolymphatic K+ concentration during ST perfusion with a solution containing 15 mmol/L K+. Perfusion of the

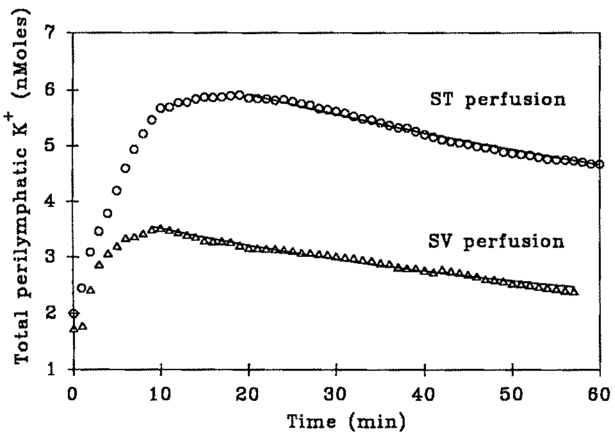

Fig 5. Calculated total perilymphatic potassium (K^+) content for 1-mm segment in second turn based on scala tympani (ST) and scala vestibuli (SV) perfusion data in Figs 2 and 3. Areas used for ST and SV were 0.18 mm^2 and 0.21 mm^2, respectively.[10] Note that for either case, clearance of K^+ after perfusion is relatively slow. Lines show fitted exponential curves with clearance half-times of 71 and 37 minutes for ST and SV perfusion, respectively. This clearance is presumed to be directed toward vasculature.

ST was chosen, as in this condition the K^+ concentrations are elevated in both the ST and SV, in contrast to the situation for SV perfusion, in which the ST is little changed. Figure 4 illustrates the typical responses of the EP and endolymph K^+ to this treatment. The EP shows a substantial elevation, to a value much higher than that usually recorded in the second turn. The EP value peaked some time after the perfusion stopped and declined a little, but remained above the preperfusion value. The endolymph K^+ concentration did not show any systematic change associated with the K^+ elevation of perilymph.

The degree of K^+ clearance to blood was estimated by calculating the total perilymphatic K^+ content for a 1-mm segment of the second turn for the ST and SV combined. Figure 5[10] illustrates the calculated total perilymphatic K^+ content for the SV perfusion (data from Fig 2) and ST perfusion (data from Fig 3) experiments. In each case the amount of K^+ increased during the perfusion period and fell only slowly after perfusion. The decline was approximated by an exponential curve returning to the preperfusion value. The least-squares curves fitted to the data represent clearance with half-times of 71 minutes for ST perfusion experiments and 37 minutes for SV perfusion experiments.

DISCUSSION

In this study we have observed two significant findings that must be considered. The first is that in spite of rapid cross-communication of small solutes across the spiral ligament, we confirmed that the SV has a physiologic K^+ concentration approximately twice that of the ST. The second is that we have demonstrated there is rapid clearance of K^+ from the ST that appears directed toward the SV.

The most likely explanation of these findings is that K^+ movements are related to the known circulating currents associated with cochlear transduction. It is generally accepted that this current is carried through the hair cells predominantly by K^+. This raises the question whether circulating currents are of sufficient magnitude to give the effects reported here. In our experiments, the maximal rate of K^+ entry into the SV was 1.36 mmol/L per minute, which occurred 5 minutes after the onset of ST perfusion (Fig 3). Assuming an SV volume of 0.21 mm^3 for a 1-mm segment in the second turn,[11] this represents a K^+ flux of 2.86×10^{-10} mol/mm per minute. The magnitude of the circulating current was estimated by Johnstone and Johnstone[12] to be 3.1 to 6.2 μA for a 2-mm segment of the basal turn, corresponding to 1.5 to 3.1 μA/mm. On the basis of current density measurements in the second turn, Zidanic and Brownell[13] estimated the total circulating current to be 0.8 to 1.6 μA/mm, of which approximately 0.4 to 0.8 μA/mm passed through Reissner's membrane.[14] By using Faraday's constant (96,500 coulombs/mol), it can be calculated that a K^+ current of 0.5 μA/mm crossing Reissner's membrane would represent a K^+ flux of 3.1×10^{-10} mol/mm per minute. This is closely comparable to our estimated flux. An alternate approach is provided by radiotracer turnover studies, from which somewhat lower fluxes can be inferred. Konishi et al[10] reported a half-time of 55 minutes for K^+ in endolymph of the basal turn of guinea pigs. Sterkers et al[15] estimated the rate constant for rat endolymph to be 0.0114 $minute^{-1}$, which corresponds to a half-time of 61 minutes. Assuming that the total K^+ content of a 1-mm segment of endolymph is 1.6×10^{-8} mol (segment volume 0.1 μL, K^+ concentration 160 mmol/L), this turnover rate would represent a K^+ efflux of 2.0×10^{-10} mol/mm per minute, of which approximately half may be passing through Reissner's membrane. This is lower than our figure, but is of the same order of magnitude and clearly shows that the K^+ movement into the SV could be accounted for by the circulating current. It is possible that the magnitude of the circulating current may be changed by high-K^+ perfusion of the perilymphatic spaces, although the interrelationships are complex. Elevated perilymphatic K^+ will tend to reduce the gradient for K^+ efflux from endolymph, while the K^+-induced elevation of the EP will tend to increase the efflux. The combined effects are small with respect to the normal driving gradient, so changes in K^+ flux are not likely to be markedly different from those calculated here.

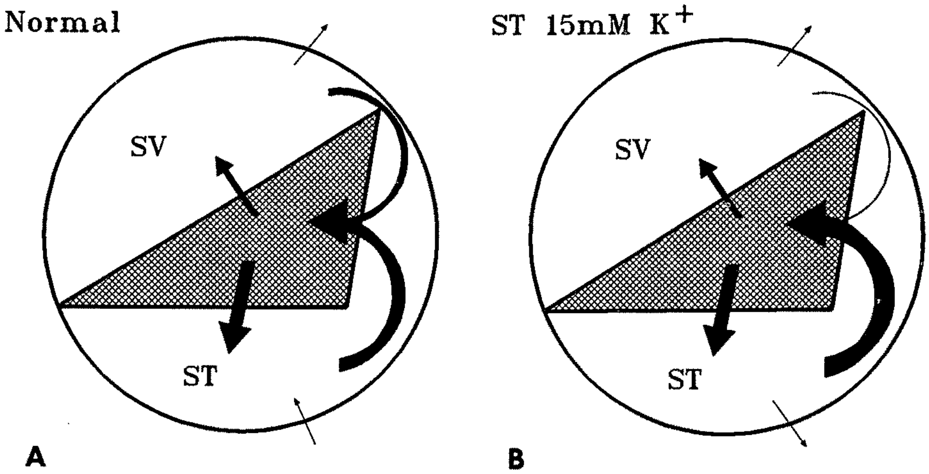

Fig 6. Schematic interpretation of experimental findings. A) In normal cochlea, potassium (K^+) in scala tympani (ST) has more ready access to stria vascularis (SV). This would account for lower physiologic K^+ concentration of ST perilymph. B) When ST K^+ is increased, greater proportion of K^+ is taken from ST and lower proportion is taken from SV. This would result in clearance of K^+ from ST and apparent accumulation of K^+ in SV.

A second requirement for the observed findings is that the K^+ transported into the scala media must be taken predominantly from the ST. This is supported by the observation that after ST perfusion, total perilymphatic K^+ was relatively stable for 10 minutes after perfusion (Fig 6). During this time the amount of K^+ taken from the ST must approximately equal the amount entering the SV. Such a preferential uptake of K^+ could occur if K^+ in the ST has greater access to the spiral ligament and stria vascularis than does K^+ in the SV. When the ST K^+ concentration is greater than the SV concentration (as it is 5 minutes after ST perfusion onset; Fig 3), a major proportion of K^+ must be taken from the ST side. This phenomenon can be readily explained by the anatomy of the lateral wall of the cochlea. In the second turn, the spiral ligament at the level of Reissner's membrane is very narrow relative to the wide communication with the ST. If this portion of the spiral ligament provides the limiting resistance for SV-ST communication, then only when the ST has a lower K^+ content than the SV (as in the normal state) will there be a net flux of K^+ from the SV directed toward the ST, which may be taken up by the stria vascularis. In the experimental situation in which the ST has a higher K^+ content than the SV, the net flux would be directed toward the SV, so it is possible that little or no K^+ would be taken up by the stria from the SV side. The salient features of this interpretation are summarized in Fig 6. In this explanation clearance from the ST is part of the circulating current directed toward the endolymph. The increased amount taken from the ST appears to be balanced by a reduced uptake of K^+ from the SV, resulting in a net accumulation there.

The proposal that K^+ has a greater access to the stria vascularis from the ST side may also provide the explanation for the difference in physiologic K^+ concentration between the ST and SV in the normal state. Even with preferential K^+ uptake from the ST, an important factor influencing the steady state concentrations would be the passive communication properties of the spiral ligament. Previous studies with TMPA as a volume marker have shown rapid cross-communication with a half-time of approximately 20 minutes. As K^+ has a higher diffusion coefficient than TMPA, passive K^+ cross-communication could be expected to be even faster than this rate and will tend to equilibrate the concentrations in the two scalae. For an ST half-time of 20 minutes and assuming ST and SV concentrations of 3.5 and 7 mmol/L, respectively, it can be calculated that the net K^+ passive flux toward the ST would amount to 2.18 x 10^{-11} mol/mm per minute. This flux is very small (approximately 1%) relative to the circulating current, so that K^+ distribution across the spiral ligament will be dominated by the circulating current. A further possibility that must be considered is that uptake of K^+ preferentially from the ST side may not have passive origins, but may be performed actively. Schulte and Adams[16] have shown that the spiral ligament on the ST side contains high levels of Na- and K^+-activated adenosine triphosphate, the function of which is not yet known. It is possible that these cells are involved in actively sequestering K^+ from the ST perilymph.

A physiologic consequence of a preferential uptake of K^+ from the ST is that the ST perilymph K^+ composition would be extremely well regulated and excess K^+ would be cleared at a very rapid rate, as demonstrated in the present study. In a previous study we reported K^+ clearance from the ST after high-K^+ perfusion with a half-time of 5.9 minutes.[5] This compared well with the present observations in which an ST clearance half-time of 7.2 minutes was observed. Although it was originally assumed that this clearance was directed to blood, it is now apparent that most of the clearance is in fact directed toward the endolymph, and from there to the SV.

The degree of communication for K^+ between perilymph and blood was estimated, with the assumption that endolymph volume and K$^+$ concentration were unchanged by the experimental treatment. An increase in endolymph K$^+$ concentration is highly unlikely because of osmotic considerations, but we cannot be certain that the endolymph volume remains unchanged. However, the clearance rates of 37 minutes (SV perfused) or 71 minutes (ST perfused) are comparable with the rate of constant for K$^+$ of 0.020 minute^{-1} estimated for the perilymph of rats (half-time 34 minutes).[15] The estimated clearance also compares favorably with the half-times of 45 to 55 minutes found by Jung et al[17] in perilymph for small ions such as K$^+$. Thus, there is no indication that K$^+$ accumulates in endolymph during perfusion. The communication with blood also represents a minor process compared to the circulating current magnitude. The previously calculated total circulating K$^+$ flux of 6.2 x 10^{-10} mol/mm per minute represents a turnover half-time of 2.7 minutes for the perilymphatic compartment in the second turn. This is somewhat faster than the perilymph K$^+$ rate constant of 0.08 (half-time 8.6 minutes) measured in rats.[15] It can be concluded that the K$^+$ turnover of perilymph with respect to blood is slower than that with respect to endolymph. This conclusion is in agreement with the concept that K$^+$ is recycled between the perilymph and endolymph rather than being drawn from blood. This concept was initially based on the observation that the EP declines rapidly when the perilymphatic space is perfused with K$^+$-free medium,[18] while the EP is maintained when the cochlear vasculature is perfused via the cannulated basilar artery with K$^+$-free artificial blood.[19] It is apparent that the magnitude and physiologic characteristics of the circulating current are sufficient not only to influence, but to dominate the kinetics for K$^+$ in perilymph.

The observed clearance of K$^+$ toward the SV is relevant to pathologic conditions that affect the cochlear fluids, such as Meniere's disease. It has been speculated that auditory dysfunction, including threshold elevation and tinnitus, may result from the release of high-K$^+$ endolymph into the perilymphatic spaces. Our data show that in the event of a release of K$^+$, changes in the ST are likely to be small, since K$^+$ is rapidly cleared. Certainly, levels may not reach those that induce motile responses of the hair cells in vitro, although it is possible that the hair cells are more sensitive to K$^+$ changes in vivo. Further examination of this hypothesis may be required. On the other hand, release of K$^+$ into the perilymph of the ST or SV will certainly result in an accumulation in the SV, as reported here. This is especially true if the release of K$^+$ is of a diffuse nature, rather than a membrane rupture. Subsequent diffusion of accumulated K$^+$ from the SV into the vestibular perilymph would be expected to disturb vestibular function and may contribute to the vestibular symptoms associated with Meniere's disease. The possibility of K$^+$ accumulation in the SV and the perilymph of the vestibule becomes even stronger as hydrops develops. Histologic examination of hydropic cochleas shows that Reissner's membrane may often be so distended as to contact the wall of the SV.[20] In this situation the return path for K$^+$ that has left the endolymph through Reissner's membrane will be occluded, and possibly high levels of K$^+$ may develop in the vestibular perilymph. This possibility merits further investigation.

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6TH INTERNATIONAL CONGRESS ON INTERVENTIONAL ULTRASOUND

The 6th International Congress on Interventional Ultrasound will be held Sept 7-10, 1993, in Copenhagen, Denmark. For further information, contact Christian Noisse, Congress Secretary, Department of Ultrasound, Herley Hospital, University of Copenhagen, DK-2730 Herley, Denmark.

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4TH INTERNATIONAL ACADEMIC CONFERENCE IMMUNOBIOLOGY IN OTOLOGY, RHINOLOGY AND LARYNGOLOGY

The 4th international academic conference Immunobiology in Otology, Rhinology, and Laryngology will be held in Oita, Japan, April 4-7, 1994. For further information contact Goro Mogi, MD, Dept of Otolaryngology, Oita Medical University, Hasama-machi, Oita 879-55, Japan; telephone 81-975-49-4411 ext 2912 or 2913; fax 81-975-49-0762.

Ann Otol Rhinol Laryngol 102:1993

IMAGING CASE STUDY OF THE MONTH

INTERNAL CAROTID ARTERY SIMULATING A MIDDLE EAR MASS

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BRASÍLIA, BRAZIL

CASE REPORT

A 32-year-old woman was referred to one of the authors (C.A.O.) in September 1990. She complained of pain in the left ear of 4 years' duration. The pain was described as dull and constant, without any pulsatile feeling. She denied tinnitus very definitely and was aware of mild hearing loss in the left ear. There were no symptoms referable to the vestibular system and her general health was good. Physical examination showed a mass in the middle ear occupying the left hypotympanum and bulging against the tympanic membrane up to the level of the umbo. No pulsations could be seen in the mass even at 16x magnification on the operating microscope, and it looked reddish at otoscopy. An audiogram showed only a slight conductive hearing loss in the left ear (10 to 15 dB air-bone gap). At tympanometry pulsation could not be detected in the left ear even at the maximum sensitivity level of the equipment. A computed tomography (CT) scan done elsewhere without intravenous contrast showed a mass in the left hypotympanum that was interpreted as a glomus tympanicum tumor, so the patient was referred to the otolaryngology department for surgery. In reviewing the CT scan the senior author (W.E.S.) noticed gross asymmetry between the carotid canals, so he decided to repeat the CT scan with intravenous contrast. This examination is shown in Figs 1 through 3. The left internal carotid artery followed an abnormal course and actually entered the hypotympanum, reaching the level of the promontory in the middle ear (Fig 1E,F). The left internal carotid canal was much smaller

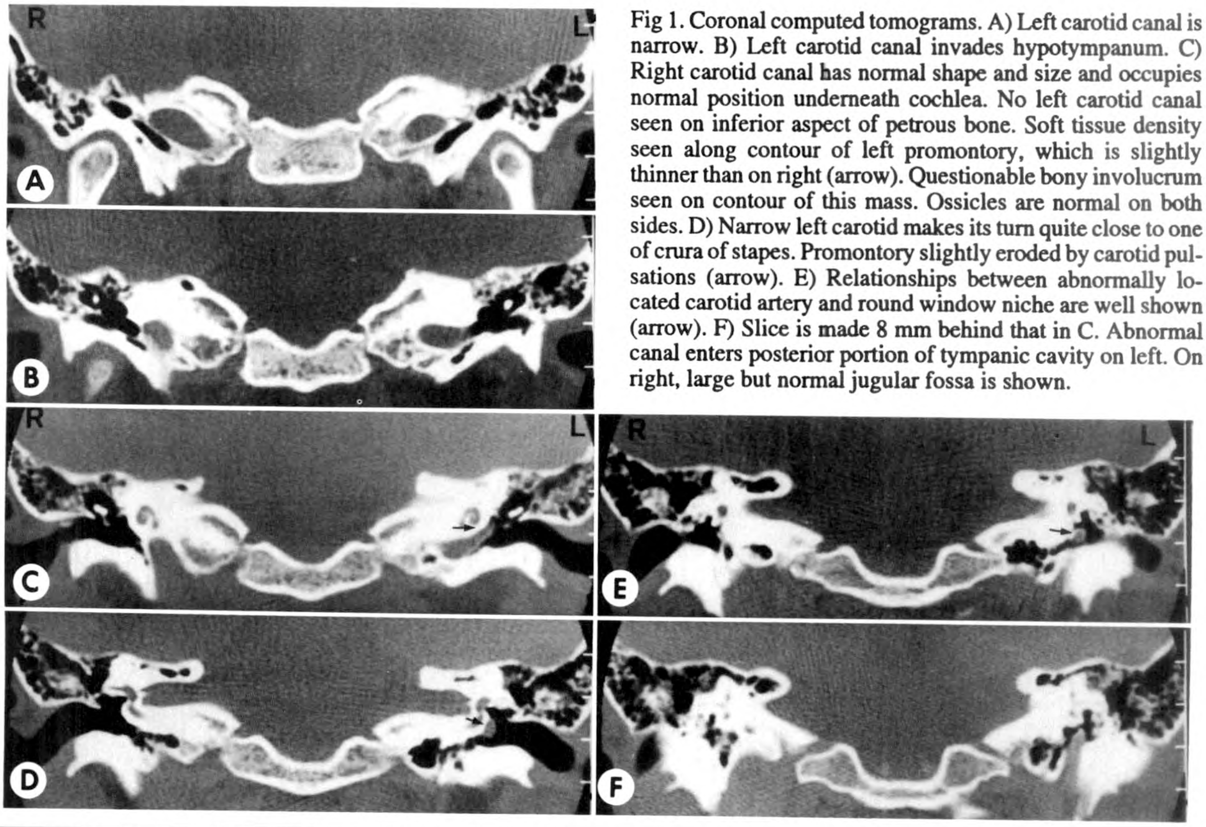

Fig 1. Coronal computed tomograms. A) Left carotid canal is narrow. B) Left carotid canal invades hypotympanum. C) Right carotid canal has normal shape and size and occupies normal position underneath cochlea. No left carotid canal seen on inferior aspect of petrous bone. Soft tissue density seen along contour of left promontory, which is slightly thinner than on right (arrow). Questionable bony involucrum seen on contour of this mass. Ossicles are normal on both sides. D) Narrow left carotid makes its turn quite close to one of crura of stapes. Promontory slightly eroded by carotid pulsations (arrow). E) Relationships between abnormally located carotid artery and round window niche are well shown (arrow). F) Slice is made 8 mm behind that in C. Abnormal canal enters posterior portion of tympanic cavity on left. On right, large but normal jugular fossa is shown.

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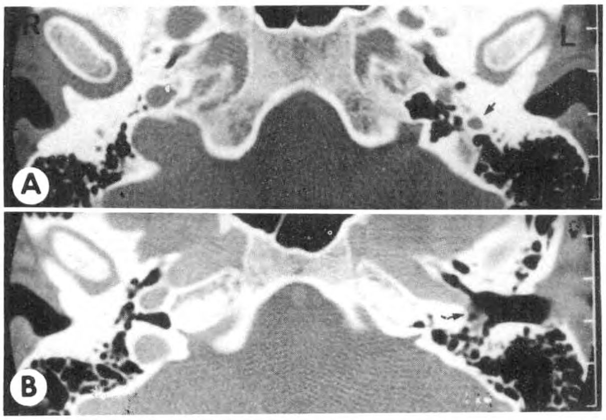

Fig 2. Axial computed tomograms. A) Small left carotid canal (arrow) is located 8 mm behind plane of normal right one. Normal anatomic relationships between carotid canal and jugular fossa are shown on right side. On left side small jugular fossa is seen on same sagittal plane as small carotid canal, but in slightly medial position. B) Abnormal course of left carotid canal in tympanic cavity, and its small caliber, are well demonstrated (arrow). Right carotid canal is only partially seen in this plane.

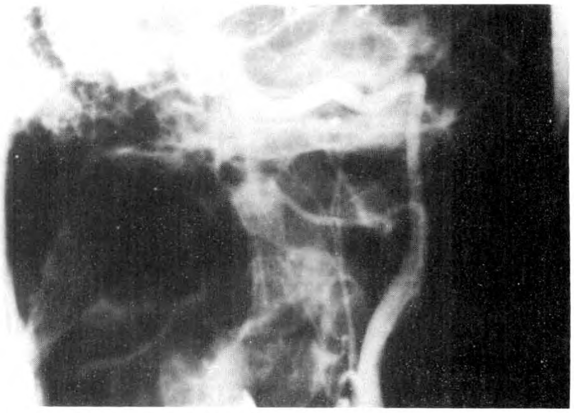

Fig 4. Left common carotid angiogram in Stenvers projection confirms both narrowing of left internal carotid artery and its abnormal course in middle ear along contour of promontory. First segment of anterior cerebral artery is hypoplastic (normal variant). No abnormalities were seen on external carotid artery branches.

than the right one but was still patent (Fig 3A,B). A carotid arteriogram (Fig 4) confirmed the CT scan findings and showed a narrow left internal carotid artery coursing through the hypotympanum of the left ear. Because controllable pain was the only symptom, we decided not to operate on this patient. She is being followed up closely and her clinical picture has not changed so far (15 months of follow-up).

DISCUSSION

An aberrant carotid artery entering the middle ear has been described in the past as a pulsatile middle ear mass.[1-3] The differential diagnosis between a middle ear vascular tumor (mainly glomus tympanicum tumor) and an aberrant internal carotid artery entering the hypotypanum was not easy because of the limitations of imaging techniques. In fact, several patients were taken to the operating room with the wrong diagnosis, and life-threatening bleeding resulting from biopsy of the artery was the first sign of the right diagnosis, which was then confirmed by arteriography.[2,4]

Since the introduction of polytomography in medical practice, the comparison of tomographic sections of temporal bones with slices of the same bones after decalcification has made the radiographic anatomy of the temporal bone well known to us.[5] Because of the marked asymmetry existing between the two ears, perfect positioning of the patient and meticulous attention to anatomic differences between the two sides are essential for a correct diagnosis.

The patient we describe had been examined elsewhere, and lack of attention to differences in anatomy of the carotid canals between the normal and the involved sides led to an erroneous diagnosis of mid-

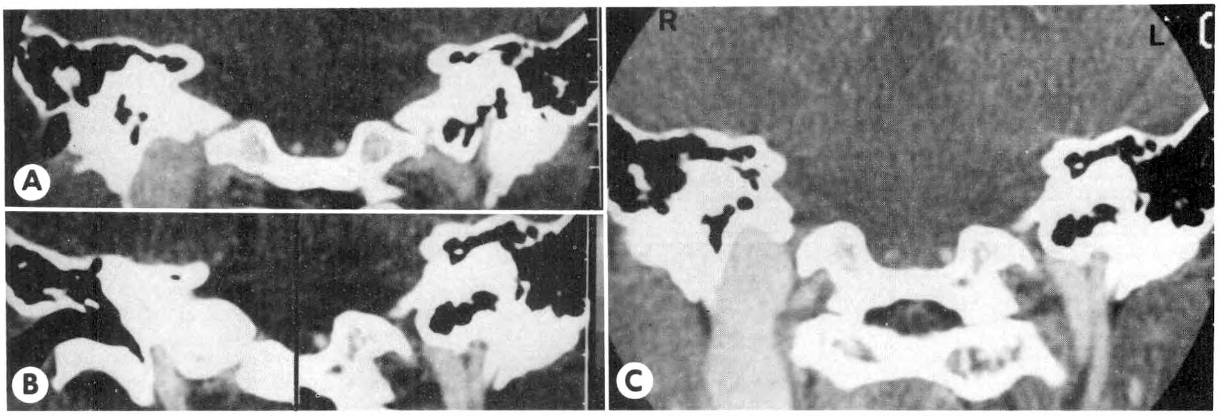

Fig 3. Contrast-enhanced coronal computed tomograms. A) Narrow left carotid artery is well enhanced by contrast medium and is seen in same anatomic plane as jugular vein. B) Composite figure of images of right and left carotid arteries. Difference between two sides is quite apparent. C) Jugular vein and carotid artery are located in same plane. Difference in diameter of two jugular veins is normal variant.

dle ear tumor. Once we noticed this detail, a repeat CT scan with blood vessel enhancement by intravenous iodinated contrast medium clearly established the correct diagnosis. Arteriography was performed just because we wanted to have a better assessment of the entire carotid system.

The lack of pulsatile tinnitus and the fact that no pulsation could be detected at otomicroscopy or at tympanometry are probably explained by the fact that the left internal carotid artery was much narrower than the right and therefore blood flow in the left was probably reduced. One can speculate that the vessel had been confined in a narrow bony space since embryonic life and therefore could not develop fully. There has been no previous report of a narrowed aberrant internal carotid artery in the hypotympanum. Why only in the last 4 years this patient has had pain in the left ear, since this is a congenital lesion, is difficult to explain.

In conclusion, when an abnormal course of the petrous internal carotid artery is suspected, the imaging technique of choice is a CT scan with intravenous iodinated contrast enhancement in the dynamic mode. The stored raw data are used for reconstruction of images with window settings for bone and soft tissue structures. Invasive methods such as arteriography should therefore not be necessary to establish the diagnosis of this congenital abnormality.

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THIRD INTERNATIONAL SYMPOSIUM ON MENIERE'S DISEASE

The Third International Symposium on Meniere's Disease will be held October 20-23, 1993, in Rome, Italy. For information, contact Kugler Publications bv, Congress Department, PO Box 1498, New York, NY 10009-9998; telephone (212) 477-1970; fax (212) 477-0181.

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MASTERS IN OTOLARYNGOLOGY

The University of Pittsburgh otolaryngology faculty will be holding its Continuing Medical Education Program March 13-20, 1993, at the Sheraton Steamboat Resort in Steamboat Springs, Colorado. For additional information, contact Robin Wagner, The Eye and Ear Institute, 203 Lothrop St, Pittsburgh, PA 15213; phone 412-647-8783; fax 412-647-8720.

Ann Otol Rhinol Laryngol 102:1993

PATHOLOGY CONSULTATION

SOLITARY FIBROUS TUMOR

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First described as a pleural neoplasm, the solitary fibrous tumor has been reported in a number of extrapleural sites, including the upper respiratory tract. The neoplasm is of mesenchymal origin, exists in benign and malignant forms, and is a histopathologic diagnosis made after exclusion of other soft tissue neoplasms. None of the eight reported sinonasal and nasopharyngeal solitary fibrous tumors have been histologically or biologically malignant. Surgical excision appears to control the tumor at these sites.

It is one of the curiosities of surgical pathology that a diagnostically difficult and biologically controversial lesion, seemingly specific for and limited to a defined anatomic site, is sometimes transposed, with all its clinical and pathologic "baggage," to unrelated organs or tissues. The solitary fibrous tumor is a case in point. First described as a primary spindle cell tumor of the pleura,[1] it belongs to a group of neoplasms that show no evidence of mesothelial differentiation by light-optic, immunocytochemical, or electron-optic studies. The tumor's cells have been defined as mesenchymal (fibroblastic or myofibroblastic).[2]

What has been described as a "recent surge in interest in these tumors"[2(p177)] of the pleura has been followed by the reporting of similar lesions in such diverse extrapleural locations as lungs, peritoneum, thymus, liver, and sinonasal tract.[3-5] In these sites, pathologists are told that the diagnosis is "based on the lesion having a light microscopic appearance characteristic of this entity and different from other neoplasms that occur in the same region."[4(p846)] Neither of these inspire confidence, especially since solitary fibrous tumors of the pleura are *characterized* by a multiplicity of histologic patterns and an absence of a single, distinctive morphologic growth pattern.[2] For those tumors in the pleura it is stressed that close attention to location and gross morphologic features is essential for proper identification of the histologic findings — a wise admonition for a tumor whose histologic appearance was once described as patternless.[2]

The solitary fibrous tumor does, however, have two fundamental architectural features: solid spindle and diffuse sclerosing.[2] Both are found in all tumors in various proportions. A dominance of a stromal fibrous component over cellular elements typifies the sclerosing forms of the tumor. A diffuse hyalinization may be so extensive that cellular or parvicellular zones are seen. In other instances, the sclerosis is less uniformly displayed, so that cellular areas are readily identifiable. The solid type also has a variety of subarchitectures that in turn recall 1) vascular tumors such as hemangiopericytoma or angiofibroma, 2) neurogenous neoplasms, 3) sarcomas such as fibrosarcoma or monomorphic synovial sarcoma, or 4) various types of fibrous histiocytomas.

Up to 23% of pleural solitary fibrous tumors have manifested an aggressive clinical behavior. This is expressed by local invasion and recurrence, although distant metastases have occasionally been documented.[2-4] The histologic appearance does not allow for complete prognostication, but aggressiveness is usually associated with tumors of large size, hypercellularity, increased mitoses, pleomorphism, and necrosis — all markers of a sarcoma. Resectability is the most important prognostic factor.[4]

On the basis of a histologic and immunocytochemical similarity to counterparts in the pleura, eight solitary fibrous tumors of the upper respiratory tract have been reported.[4,5] All have been in the sinonasal tract or nasopharynx. Five have been in women; three in men. The mean age of the patients at the time of diagnosis was 48 years (range 30 to 64 years). The tumor occupied the nasal cavity in every patient except one, whose lesion was said to be

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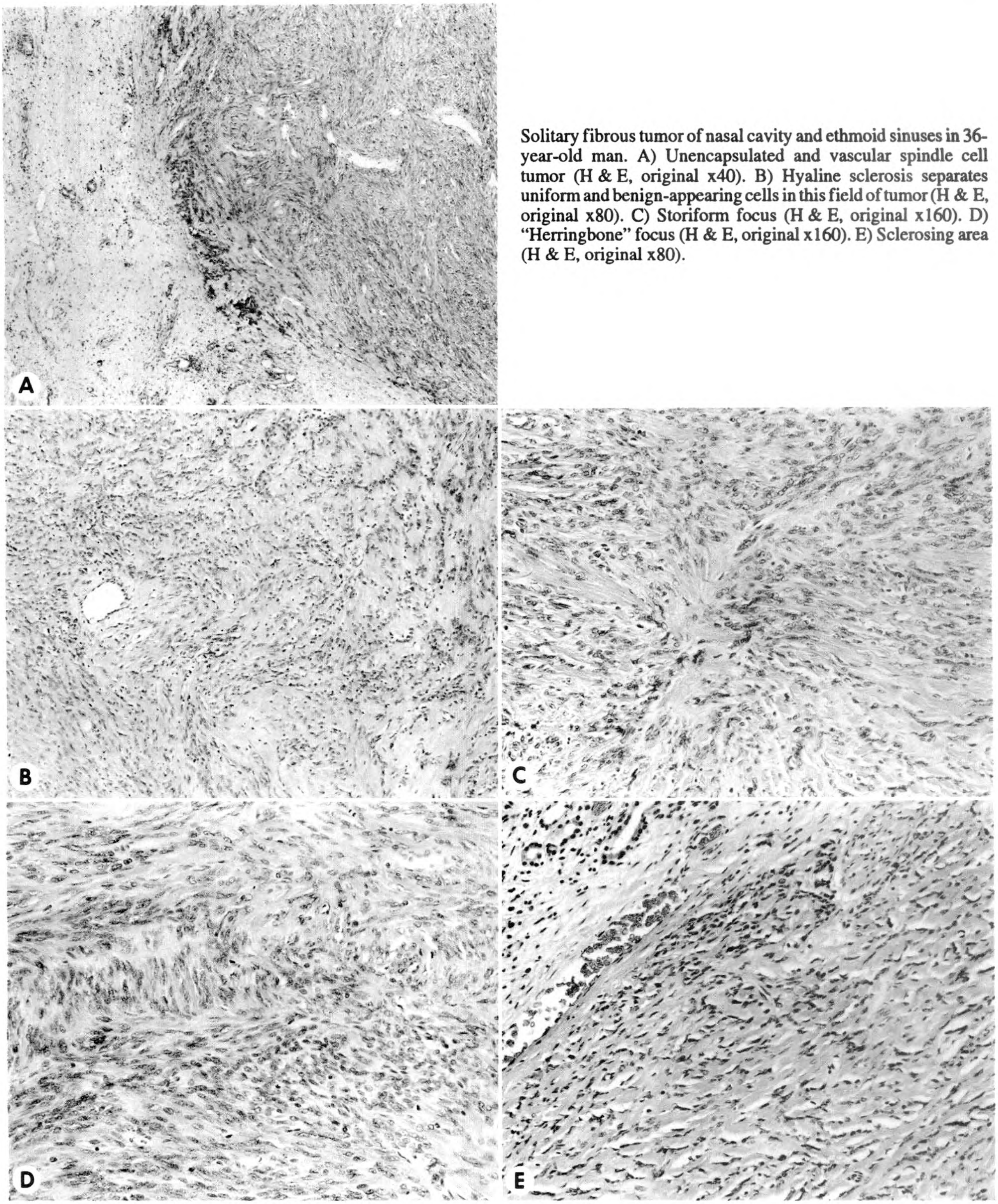

Solitary fibrous tumor of nasal cavity and ethmoid sinuses in 36-year-old man. A) Unencapsulated and vascular spindle cell tumor (H & E, original x40). B) Hyaline sclerosis separates uniform and benign-appearing cells in this field of tumor (H & E, original x80). C) Storiform focus (H & E, original x160). D) "Herringbone" focus (H & E, original x160). E) Sclerosing area (H & E, original x80).

limited to the nasopharynx. Two patients had nasal cavity and nasopharyngeal involvement, and extensions to the ethmoid sinuses were manifested by the nasal tumors in two patients.

Gross descriptions have been sketchy, aside from the fibrous, firm quality of the lesions. Four tumors were described as "polyps" in the nasal cavity. Two others filled or partially filled the affected side of the nasal cavity, and each also extended into the ethmoid sinuses. Two tumors were inadequately described.

With light-optic examination the tumors, like the one illustrated in the Figure, were unencapsulated and showed nearly all of the histologic findings ascribed to their pleural counterparts, ie, haphazard proliferation of spindle cells in a collagenous background, variable cellularity within the tumor, a prominent vascular pattern, and an immunoreactivity only with antibodies against vimentin. Necrosis, hemorrhage, and mucosal ulceration have been absent, and mitoses few in number.

All patients had their neoplasms resected by surgical procedures that ranged from "local resection" to medial maxillectomy with sphenoidectomy or ethmoidectomy and sphenoidectomy. Follow-up (6 months to 12 years) was available for seven of the eight reported patients. Only one patient has had persistence (stable over 4 years) after local resection.

We accept the premise that a histologic equivalent of solitary fibrous tumor of the pleura occurs in the upper airway. Indeed, the sinonasal tract and nasopharynx seem to be preferred sites for these extrapleural tumors. To date, none of the tumors have been histologically or biologically malignant, but the number of acceptable reported cases is small.

The danger of a "novelty diagnosis" intrudes in the histopathologic evaluation of these tumors. The differential diagnosis is such that a diagnosis of solitary fibrous tumors outside the pleura is almost one of exclusion. Foremost in the exclusion list are hemangiopericytoma, neurogenous neoplasms, and fibrous histiocytoma.

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OBJECTIVE MEASUREMENTS OF INNER EAR FUNCTION AND DYSFUNCTION

The Objective Measurements of Inner Ear Function and Dysfunction: Sixth Annual Electrocochleography (ECoG)/Otoacoustic Emissions (OAE) Seminar and Video Workshops will be held February 17-20, 1993, in Denver, Colorado. For further information, contact Apryl Salz or Jane Wells, Meeting Coordinators, c/o I. Kaufman Arenberg, MD, FACS, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; phone 303-850-9545; fax (303) 788-4234.

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NEW PERSPECTIVES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

This international symposium, to be held July 5-8, 1993, at St Thomas's Hospital, London, England, will be sponsored by Harvard Medical School, Beth Israel Hospital–Brigham & Women's Hospital, Guy's and St Thomas's Medical and Dental School, and the Université René Descartes Faculté de Medicine, Necker Enfants Malades, Hopital Laennec. For further information, contact Harvard Medical School, Department of Continuing Medical Education, PO Box 825, Boston, MA 02117; phone 617-432-1525.

LETTERS TO THE EDITOR

To the Editor:

In the recent paper by Campbell et al (Interpretation of Electrocochleography in Meniere's Disease and Normal Subjects, *Ann Otol Rhinol Laryngol* 1992;101:496-500), a question is raised as to the clinical utility of electrocochleography (ECochG) based upon the authors' comparison of a group of only 10 patients with Meniere's disease to a group of 20 normal volunteers. Having had the benefit of using ECochG in thousands of patients over the past 10 years, utilizing both the Coats canal electrode and a transtympanic approach, we believe a response to this paper is in order.

As mentioned, this study is based upon an extremely small sample of Meniere's patients. It is further complicated by the fact that only 1 in 10 reported aural fullness, a symptom that we find in almost every case of Meniere's disease. Further, the audiometric data reported make one wonder if all 10 patients really had Meniere's disease. Audiometric thresholds are only reported for 2,000, 4,000, and 8,000 Hz. No low-frequency information is provided, where one would expect to see a rising or flat audiometric configuration. Moreover, 3 of 10 subjects clearly demonstrate a falling audiometric pattern from 2,000 Hz to 8,000 Hz, which is atypical for Meniere's disease.

Finally, the extreme variability in amplitude for the action potential and summating potential measurements in both groups clouds this study and reflects the well-documented problem with ECochG recordings made in the ear canal or on the tympanic membrane. In our experience, as well as that of others,[1-3] transtympanic ECochG provides much higher-amplitude recordings, which are also less variable. In addition, the separation between patients with Meniere's disease and normals, as well as those with other sensorineural hearing losses, is quite clear-cut.

We believe the present study does not represent the typical experience of some clinicians with ECochG, especially transtympanic ECochG, and that it unfairly criticizes a valuable clinical tool.

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To the Editor:

Thank you for allowing me the opportunity to respond to Dr Shea's letter regarding our article. I too was disappointed that electrocochleography (ECochG) did not clearly differentiate between the normal subjects and Meniere's disease patients in our study. Nonetheless, the data were carefully collected and analyzed and do have clinical implications.

Dr Shea is incorrect in suggesting that our Meniere's disease patients were not selected according to conservative diagnostic criteria. Our criteria for selection are delineated in the methods section, and are as, or more, restrictive than those listed in other published studies. We did not include borderline or questionable cases.

It is not true that only one patient had aural fullness. Table 3, as indicated in the heading and the text, lists *symptoms on the date of assessment.* Meniere's disease symptoms are inherently fluctuant and/or intermittent. We analyzed the data according to symptoms on the date of assessment because Ferarro et al[1] suggested that this type of analysis may improve the hit rate of ECochG in the detection of Meniere's disease. This does not suggest that only one of these patients ever had aural fullness, and indeed that was not the case. In fact, two of the patients had aural fullness on the date of assessment, as listed in Table 3, and intermittent aural fullness was very common in these patients, particularly in conjunction with an attack. Patients are usually too ill to attend an appointment or tolerate testing during an acute episode of the disease.

The audiometric thresholds are listed only for 2,000, 4,000, and 8,000 Hz because considering the duration and frequency components of our stimuli, the ECochG response would be expected to correlate most closely with this audiometric frequency range. Two of our subjects had flat audiometric configurations, one had a low-frequency hearing loss only, and seven had "cookie bite" configurations with a low-frequency loss that improved with increasing frequency and then dropped again. These seven subjects with "cookie bite" configurations include the three that Dr Shea noted as having continuously falling audiometric patterns from 2,000 through 8,000 Hz. None of these configurations are unusual for Meniere's disease, and in fact, they are quite typical.

The variability in our data was large but virtually identical to that of other published studies, as delineated and referenced in the discussion section of our paper (page 499, paragraph 1). The fact that our data are so similar to those reported in other studies of larger subject numbers would suggest that our sample is fairly typical. As referenced in the paper, our data, but not our conclusions, are similar to those of other published work, particularly for Meniere's disease patients with mild hearing loss.

I agree with Dr Shea that transtympanic ECochG may offer certain advantages over noninvasive ECochG. This possibility is discussed in the paper. The purpose of our study was to examine the various noninvasive methods and decision criteria currently proposed for a group of Meniere's disease and normal patients.

Actually, the study that needs to be done is a longitudinal study examining the summating potential and action potential in patients in whom Meniere's disease is suspected but not confirmed, with long-term follow-up to determine which patients actually developed Meniere's disease. Only this type of study could truly determine if ECochG is effective in the early diagnosis of Meniere's disease. Truly, this is the clinical question. Studies of ECochG in patients with established Meniere's disease, including my own, cannot address this issue.

Hopefully, further research will elucidate better methods to provide an early definitive diagnosis of Meniere's disease.

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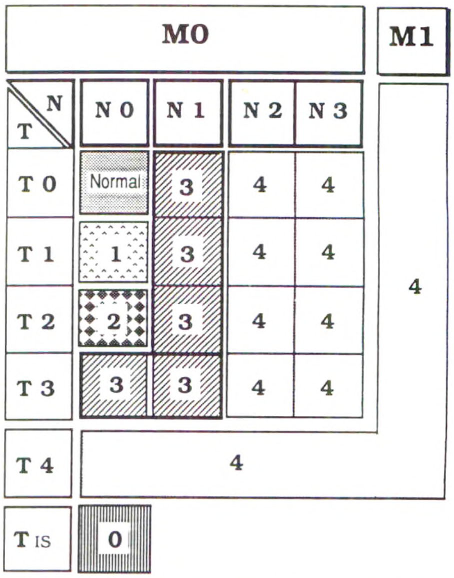
To the Editor:

Throughout my career as a professor of head and neck surgery, I noticed that some students have difficulty in remembering the TNM staging of laryngeal cancer. This stimulated me to develop the so-called MNT table (see Figure), which proved to be beneficial in teaching that system of staging. I hope that my colleagues will try it and that they find it of some help.

This table is called the MNT table because it begins with the state of distant metastasis, followed by lymph node stage versus tumor extent. It does not offer a new classification, but it is an easy way to teach students the TNM staging of laryngeal squamous cell carcinoma. This table might be applicable, with some modifications, to other head and neck or body areas.

A mere look at the table shows that if there is evidence of distant metastasis (last or M1 column), the case is stage IV irrespective of the lymph node stage or tumor extent. If the primary tumor is T4, the case is stage IV irrespective of the lymph node stage.

In the absence of distant metastasis (M0), one correlates the nodal stage (second row) with each of the tumor variables (first column) in order to assess the stage as follows. In the absence of cervical lymph node secondaries (N0 or second column), the stage will depend on the tumor extent. With T0 the case is considered normal (T0, N0, M0). With T1 the case is stage I (T1, N0, M0). With T2 the case is stage II (T2, N0, M0). With T3 the case is stage III (T3, N0, M0). With T4 the case is stage IV (T4, N0, M0). If neck examination reveals an N1 lymph node stage (third or N1 column), the case is stage III with any T except T4: T0, N1, M0; T1, N1, M0; T2, N1, M0; T3, N1, M0. If neck examination reveals an N2 or N3 lymph node stage (fourth and fifth or N2 and N3 columns) the case is stage IV with any T: T0, N2, M0; T1, N2, M0; T2, N2, M0; T3, N2, M0; T4, N2, M0; T0, N3, M0; T1, N3, M0; T2, N3, M0; T3, N3, M0; T4, N3, M0.



	M0				M1
N T	N 0	N 1	N 2	N 3	
T 0	Normal	3	4	4	4
T 1	1	3	4	4	
T 2	2	3	4	4	
T 3	3	3	4	4	
T 4	4				
T IS	0				

MNT table. M — distant metastasis, N — lymph node, T — tumor, digits — respective stages.

In the case of a metastatic cervical lymph node from an unknown primary (T0 or third row), the case is either stage III with N1 or stage IV with N2 or N3. There is no place for stages I and II, which exist only with N0. In the case of a carcinoma in situ, the lymph node stage is always N0 and the case is stage 0.

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ACKNOWLEDGMENT OF REVIEWERS

Peer review is a process aimed at ensuring high-quality professional publications. The extent to which this goal is achieved depends on the willingness of a body of reviewers to share time, experience, and abilities to assist in evaluating materials submitted for publication. It is with a great deal of gratitude that we acknowledge the persons listed below for serving the ANNALS as reviewers from November 1, 1990, through October 31, 1992. Their participation has contributed importantly to the growth and distinction of this journal.

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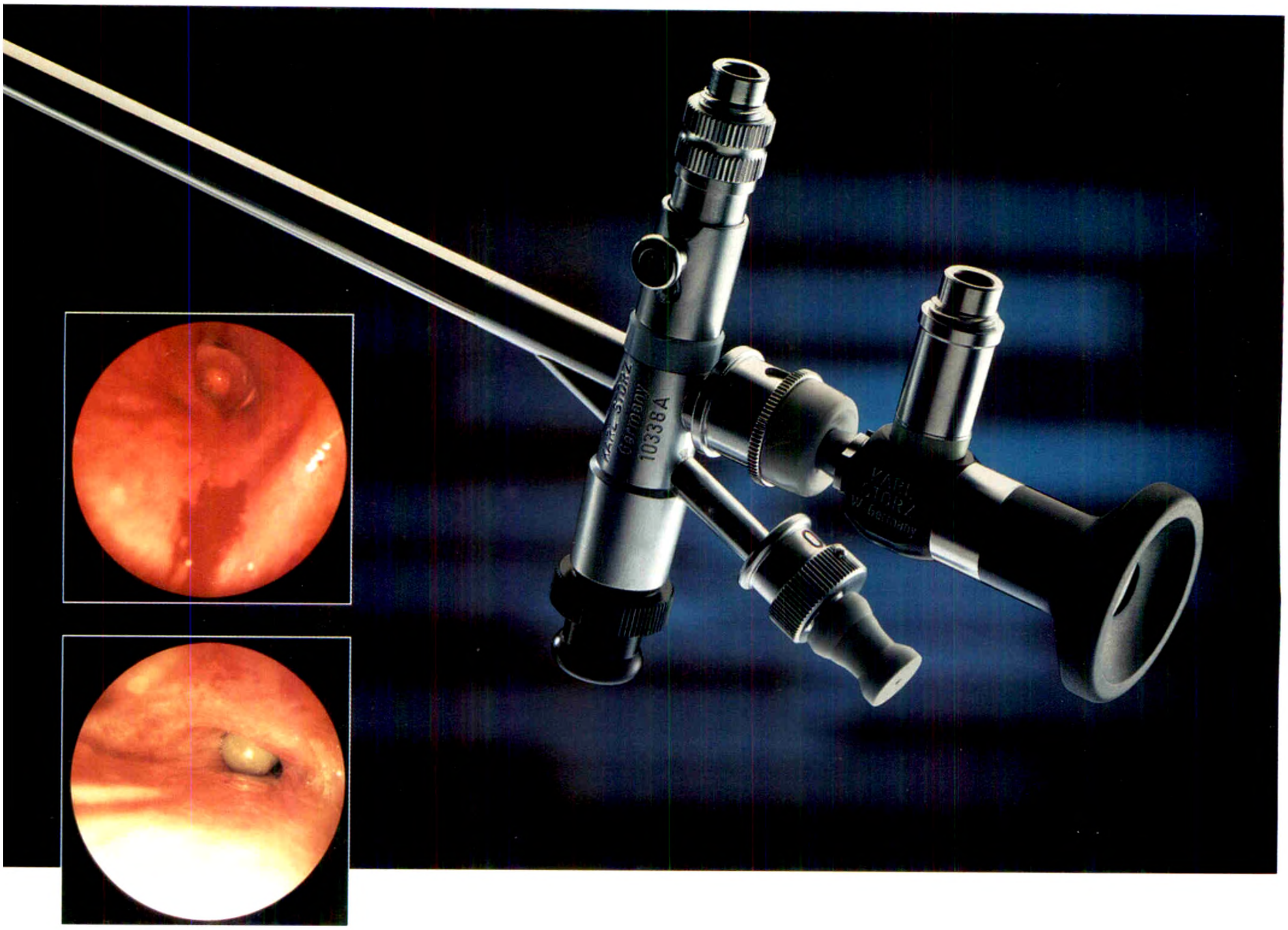

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ENDOSCOPIC LASER MEDIAL ARYTENOIDECTOMY FOR AIRWAY MANAGEMENT IN BILATERAL LARYNGEAL PARALYSIS

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A review of our recent experience in patients with bilateral laryngeal paralysis is described. While we continue to use phrenic nerve transfers in patients with mobile arytenoids, patients with fixed arytenoids generally require some sort of vocal cord lateralization, either by arytenoidectomy and arytenoidopexy or by partial vocal cord resection. The endoscopic laser medial arytenoidectomy is a convenient and effective method for opening the posterior glottic airway. One arytenoid is reduced medially with the carbon dioxide laser. After about 3 months the opposite arytenoid can be treated similarly, if necessary. The procedure does not appear to affect arytenoid mobility, as the posterior commissure mucosa and underlying interarytenoid muscle are protected and hence unaffected by the procedure. Those patients with at least one mobile arytenoid cartilage are candidates for posterior cricoarytenoid muscle reinnervation. Although ansa cervicalis and phrenic nerve techniques have been described, the author has concentrated efforts on the phrenic nerve. This report describes the endoscopic laser medial arytenoidectomy procedure, while the phrenic nerve patients will be reported in a subsequent manuscript.

KEY WORDS — airway, endoscopic laser medial arytenoidectomy, laryngeal paralysis.

INTRODUCTION

Surgeons have long sought techniques for safely widening the glottic airway in patients with bilateral vocal cord immobility and/or paralysis without detracting from vocal quality and/or causing aspiration. Over the years a variety of arytenoidectomy procedures have been described, and one or more of these have been the gold standard for many years. In the past decade the carbon dioxide (CO2) laser endoscopic arytenoidectomy has become perhaps the most commonly used method for lateralizing the arytenoid cartilage and the vocal cord in patients with bilateral paralysis or posterior glottic stenosis.

Ossoff et al[1] described a series of 28 patients managed by eight different surgeons using the CO2 laser to perform arytenoidectomy. Their technique resulted in complete ablation of the arytenoid cartilage, including both vocal and muscular processes. In addition to arytenoidectomy, their technique utilized an additional laser cut anterior to the vocal process that resulted in separation of the middle third of the cord, and the arytenoid region. This creates a triangulated posterior airway that allows for increased posterior glottic airflow. Their patients reported only a "small amount of vocal loss," which the authors attributed to the minimal lateralization of the membranous vocal cord. They stressed the heat sensitivity of the posterior commissural tissues, and felt that failure of the procedure to relieve airway obstruction in one case was due to intraoperative thermal injury to these posterior commissural soft tissues medial to the arytenoid cartilage, with subsequent scar medialization of the remaining soft tissue of the posterior vocal cord.

Lim[2] discussed 10 patients treated with endoscopic CO2 laser arytenoidectomy, using a technique similar to that described by Ossoff et al.[1] Most of these patients had glottic stenosis, not vocal cord paralysis. Nonetheless, they reported that all patients had successful return of airway, voice, and swallowing function. Vocal quality was described as fair in 5 patients, and good in the other 5.

El Chazly et al[3] described 12 patients with bilateral fixed vocal cords in whom tracheotomy decannulation was accomplished in all following endoscopic arytenoidectomy. Vocal quality was not assessed. In the German literature, Eckel[4] reported similar results in 15 cases, with 14 of the patients experiencing a satisfactory respiratory result. An innovation, gluing a mucosal flap into the endolaryngeal wound at the conclusion of the procedure, was included in Eckel's report.

Danish surgeons Greisen and Nabe-Nielsen[5] found endoscopic arytenoidectomy effective, as 23 of the 28 patients in their report were able to undergo tracheotomy decannulation; phonatory quality was not discussed.

Poch Broto et al[6] compared results of endoscopic laser arytenoidectomy with nonlaser arytenoidopexy

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and cordopexy in 14 patients with bilateral laryngeal paralysis. They concluded that the laser procedure yielded better respiratory and phonatory results than nonlaser procedures; however, phonatory quality was not critically assessed in this report.

The Finnish surgeon Rinne[7] discussed late results of laterofixation in treatment of bilateral vocal cord paralysis. In his series of 34 patients with bilateral vocal cord abductor paralysis, Woodman arytenoidopexy was performed in 31 patients. Thirteen of these 34 patients needed further surgery. Two of the 3 patients treated with endoscopic arytenoidectomy had successful outcomes. He concluded that from the initial failure rate of 22% with the Woodman procedure, twice as many patients had failed (44%) when long-term follow-up was reported, and acknowledged the trend toward endoscopic laser arytenoidectomy.

Each of these prior authors has described using endoscopic CO2 laser arytenoidectomy to enlarge the glottic airway in patients with either "glottic stenosis" or bilateral vocal cord paralysis. The goal of the procedure was in most instances tracheotomy decannulation. This goal was achieved in most cases described, indicating that the endoscopic approach to arytenoidectomy has been improved by usage of the laser, since the original Thornell endoscopic arytenoidectomy was somewhat less successful at achieving decannulation in tracheotomized patients.

Only the paper by Ossoff et al,[1] however, described in any detail the quality of voice achieved. Since it has long been accepted that any complete arytenoidectomy (particularly if there was any vocal cord lateralization [pexy], or injury to the vocal cord substance) is routinely associated with a postoperative poor phonatory result, it has been assumed that complete laser arytenoidectomy would also result in an unsatisfactory phonatory voice. The "small amount" of phonatory loss described in the Ossoff report is encouraging, since the ultimate ideal goal of any procedure for laryngeal paralysis must be normal vocal quality and function, with normal swallowing and airway function of the larynx. However, patients of Ossoff et al with complete endoscopic arytenoidectomy had moderate to severe reductions in phonatory quality.

The literature cited above largely deals with patients with severe glottic airway obstruction secondary to bilateral laryngeal paralysis and/or glottic stenosis. It seems clear from these reports that in such severe laryngeal impairment, a complete or near-complete arytenoidectomy is necessary. However, there are many other patients who, despite demonstrable airway compromise, steadfastly refuse tracheotomy. Other patients have undergone tracheotomy, but have been decannulated and left with marginal glottic airways. Many if not most of these latter patients are particularly desirous of improving their airway, yet refuse total arytenoidectomy when advised that there will be some diminution in phonatory quality. This group of patients with glottic airway impairment seek small increments of airway enhancement, but as they are often active in business and social life, they refuse complete arytenoidectomy as is described by Ossoff et al.[1]

It was this group of patients that prompted the author to seek a procedure that would improve the airway with little or no loss of laryngeal phonatory function. Reflecting on the posterior third of the glottis as the "respiratory glottis," and the anterior two thirds of the glottis as the "phonatory glottis," I reasoned that the posterior glottis could be widened by removing only the medial portion of the arytenoid body, and if the muscular and ligamentous attachments of the arytenoid were left unharmed by the procedure, the vocal process and hence the membranous vocal cord anteriorly would remain intact and in the anatomic position.

Since increased airflow would be expected not only during inspiration, but also during glottic closure (Valsalva, coughing, phonation), the effects of increasing the diameter of the respiratory glottis were difficult to predict. However, after observing in several female patients a rather large posterior glottic gap during otherwise normal phonation, it was decided that this posterior phonatory airflow between the arytenoids would not necessarily affect the voice adversely.

Since the membranous anterior glottis from the vocal process to the anterior commissure is more important to phonation than to the airway, the author felt that it might be possible to enlarge the posterior airway by partially resecting the medial portion of the arytenoid, without affecting in any way the membranous vocal cord from the vocal process to the anterior commissure (Figs 1 and 2).

MATERIALS AND METHODS

Eight patients underwent endoscopic laser medial arytenoidectomy (ELMA). Two were stridorous, with various degrees of glottic airway obstruction, but were not tracheotomized, while the other six had indwelling tracheotomies at the time of the ELMA procedure. All patients requested airway enhancement, but expressed strongly that they wanted no loss of existing vocal quality. Intrinsic muscular diseases

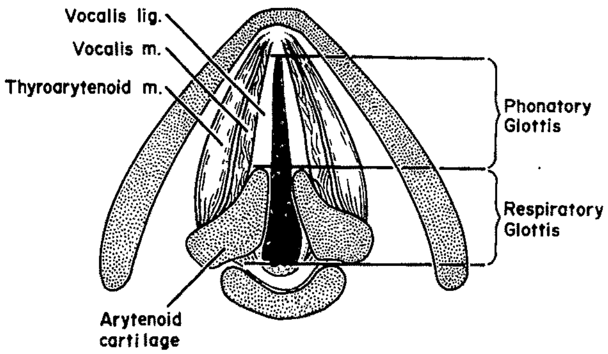

Fig 1. Axial section of larynx at glottis. Note that phonatory portion of glottis extends from vocal process to anterior commissure. Normal phonation requires symmetric vocal ligaments and muscles, and intact vocal process of arytenoid. Respiratory glottis extends from vocal process to posterior commissure.

were present in two patients, one of whom had acral muscle wasting, and bilateral abductor paralysis. The other six patients had as etiologic factors for their paralyses thyroidectomy (four) and long-term idiopathic paralysis (two).

Those patients with tracheotomies were treated with foil-wrapped or otherwise laser-protected tracheotomy tubes in place, while the two nontracheotomized patients underwent ELMA with endoscopic laser protector instruments, with ventilation via a supraglottic Venturi jet, placed either though one light port in a Pilling laryngoscope, or secured to the side of a Lindholm laryngoscope. The CO2 laser was used with a setting of from 10 to 20 W, with a spot size of 0.8 mm. I would prefer a smaller spot size for this procedure, but it was not available to me during the treatment period for these patients. Generally, I have used the pulse mode to avoid heat injury of adjacent laryngeal tissues, although occasionally it is possible to use the continuous mode for the initial (superior) portions of the medial arytenoidectomy with relative safety.

The mucosa overlying the superior and medial portions of the arytenoid is first vaporized. Then the cartilage of the arytenoid body posterior to the vocal process is slowly "shaved" with the laser, with care taken to protect the posterior commissure soft tissues with endoscopic instruments, strips of foil, or saline-soaked $^{1}/_{2}$-in cottonoid patties (Figs 1 and 2). Using a small-bore laryngeal suction tip, it is possible to accurately palpate and "map" the posterior medial corner of the arytenoid and the vocal process, and then to saucerize a concavity in the body of the arytenoid between these two points. Care must be taken not to produce laser injury inferior to the arytenoid cartilage, since this might produce cricoarytenoid joint injury, with subsequent fusion of the arytenoid to the cricoid, or further medialization of the arytenoid body secondary to soft tissue scar.

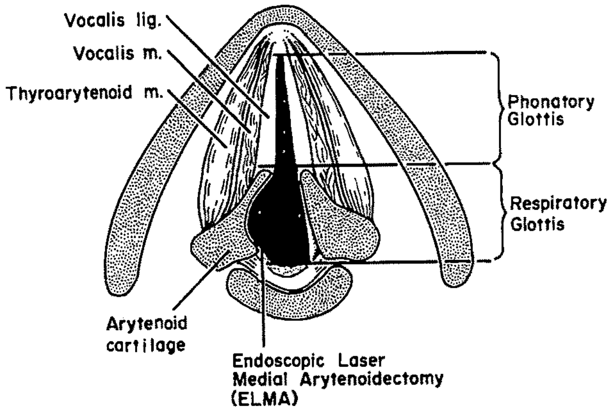

Fig 2. Endoscopic laser medial arytenoidectomy. Concavity is created along glottic edge of body of arytenoid cartilage. Vocal process and posterior commissure must be left intact and unharmed. Full superior-inferior dimension of arytenoid must be vaporized.

Hence, the goal of the ELMA procedure is an ispilateral concavity in the arytenoid body, which results in a semilunar concavity in the ipsilateral posterior glottic airway. The raw surface created in the arytenoid subsequently is covered with a thin regenerated epithelium, and the airway is generally improved by 1 or 2 mm on each side treated. Ten milligrams of intravenous dexamethasone was given intraoperatively in each case to diminish postoperative edema. A cephalosporin antibiotic was prescribed for 10 days postoperatively as well.

After a 3-month period, the procedure is repeated on the opposite side, if necessary to procure a more satisfactory airway. Since the vocal process and the remainder of the arytenoid cartilage, muscular process, and cricoarytenoid muscles are left intact, there is little or no effect of the procedure on the membranous vocal cord, unlike the other endoscopic arytenoidectomy procedures previously described.

One of my eight patients had undergone prior laryngeal reinnervation, and was thought to have arytenoid medialization secondary to synkinetic muscular imbalance between the lateral cricoarytenoid and posterior cricoarytenoid muscles. Her airway was improved by ELMA, but she subsequently sought additional airway relief. Five units of botulinum toxin (Oculinum) was injected into the lateral cricoarytenoid muscle by an electromyographic monitoring technique similar to that used in my spasmodic dysphonia patients. Airway improvement occurred in approximately 24 hours and was due to lateralization of the vocal cord and arytenoid. (Subsequently, however, she required a tracheotomy after developing laryngeal edema during a course of radioactive iodine therapy.)

Of the two patients with laryngeal airway impairment but without tracheotomies, phonatory voices were preserved and the airway was enhanced. Both patients indicated clearly that exercise tolerance was increased, and stridor decreased. Of the six tracheotomized patients, two were decannulated, one after bilateral ELMA procedures, and the other after only one procedure. The other four tracheotomized patients are awaiting a contralateral ELMA procedure (two) or have decided against further surgery (two).

DISCUSSION

Endoscopic laser medial arytenoidectomy appears to have a role in management of the compromised glottic airway, particularly in those patients with a marginal airway and no tracheotomy. In this group, the procedure offers a comparatively simple, quick, yet effective method for enhancing glottic airflow, apparently with little diminution of vocal quality. After enlarging the posterior glottic airway by 1 to 2 mm with one procedure, the operated arytenoid is allowed to reepithelialize. The procedure may then be performed on the opposite side as necessary. Although only one of my patients has had the procedure performed bilaterally, it is my opinion that approximately 3 mm of airway enhancement may be achieved by bilateral ELMA (1.5 mm per side) with little effect on membranous vocal cord position, structure, or closure.

It is, of course, possible that if too much arytenoid cartilage is resected bilaterally, aspiration could be induced. Hence, it seems cogent to laser only a conservative portion of the arytenoid whenever a second (contralateral) procedure is undertaken. However, since the literature does not indicate aspiration to be a problem when one entire arytenoid is resected, it seems unlikely that conservative bilateral medial vaporization of 1 to 3 mm of arytenoid cartilage would risk significant aspiration.

It remains to be seen whether ELMA will prove effective in only one procedure in tracheotomized patients who desire tracheotomy decannulation. Theoretically, a bilateral ELMA might yield a more normal phonatory voice than a complete unilateral arytenoidectomy. However, in the current era of cost containment, two procedures are rarely thought of (at least by third-party payors) as better than one procedure. Patients with bilateral ELMA procedures, and complete endoscopic laser arytenoidectomy should be studied in voice laboratories so that in future reports data will be objective enough to compare to other surgeons' data. However, in this initial series of patients, phonatory and pulmonary studies were not routinely completed in all patients; hence, the objective laboratory data to prove my hypotheses are lacking. I am currently evaluating all new candidates for the procedure with a full battery of phonatory evaluations such as jitter, shimmer, harmonics-to-noise ratio, and speech airflow, as well as flow-volume loop studies.

However, the sequential nature of enlarging the glottic airway 1 or 2 mm at a time appears desirable, since vocal function seems to be better preserved than with complete unilateral laser arytenoidectomy. Progressive enlargement of the posterior glottis would be expected (at some end point) to allow enough leakage of subglottic pressure between the resected arytenoids as to interfere with building of subglottic pressure (during phonation), necessary to entrain the vocal cords for normal phonatory function. However, it will entail further use of the procedure to delineate exactly how much of each arytenoid can or should be resected to achieve balance between glottic airflow during inspiration, and a sufficiently small interarytenoid gap to allow for subglottic pressure development immediately preceding and during phonation.

I have not used this procedure in previously irradiated patients. This would not seem prudent, since the irradiated cartilage might be prone to infection and/or cartilage necrosis. If a mucosal flap were created and reapplied to the resected arytenoid concavity, the procedure might be potentially safe enough for use in irradiated patients (M. Rontal, personal communication, 1992).

There were no complications in these eight patients, although one patient required subsequent tracheotomy shortly after receiving radioactive iodine.

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LASER SURGERY FOR EPITHELIAL HYPERPLASIA OF THE VOCAL FOLD

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A total of 88 patients with epithelial hyperplasia of the vocal fold were treated with endolaryngeal microscopic carbon dioxide laser surgery during the period from 1978 to 1990. The majority of the patients were male smokers. During the follow-up period ranging from 12 to 156 months, 15 patients developed recurrence(s). The recurrent lesion was benign epithelial hyperplasia in 8 patients and invasive carcinoma in 7 patients. The recurrences occurred only in preoperative smokers. Discontinuation of smoking following laser surgery did not affect the incidence of recurrence. The incidence of development of carcinoma did not differ between two histologic types: simple hyperplasia without cellular atypia and dysplasia associated with cellular atypia. The incidence of cancer development for dysplasia cases in the present series (6%) was lower than that in previous reports in which the carbon dioxide laser was not employed for the treatment. Postoperative improvement of vocal function was manifested in an increase of the maximum phonation time, a decrease of the mean airflow rate, and an increase in vibratory amplitude and mucosal wave.

KEY WORDS — epithelial dysplasia, epithelial hyperplasia, laser surgery, vocal fold.

Epithelial hyperplasia (EH) of the vocal fold has been regarded as a precancerous lesion. The value of carbon dioxide (CO2) laser surgery for EH of the vocal fold has been very well documented.[1-5] Reports on long-term results of CO2 laser surgery, however, have been limited. The vocal function following laser surgery for EH has not been well documented. Since 1978, we have been using the CO2 laser for the treatment of EH of the vocal fold at Kurume University Hospital. The purposes of this paper were to investigate the long-term results of CO2 laser surgery and to evaluate postoperative vocal function.

SUBJECTS AND METHODS

During the period from 1978 to 1990, a total of 88 patients with EH of the vocal fold underwent endolaryngeal microscopic CO2 laser surgery in Kurume University Hospital. Of the 88 patients, 80 were men and 8 were women. Their ages ranged from 35 to 82 years with a mean of 59 years. Fifty-eight patients had unilateral lesions (39 on the right and 19 on the left), whereas 30 had bilateral lesions.

Distribution of the 88 patients by preoperative history of cigarette smoking is shown in Table 1. The Brinkman index, which is defined as the number of cigarettes per day multiplied by the duration of smoking in years, was employed as the parameter for the degree of smoking. Approximately two thirds of the patients were heavy smokers (Brinkman index > 400). The majority (69 of 80 or 86%) of the men were smokers, whereas only 1 of the 8 women was a smoker and she had a low Brinkman index.

The surgery was conducted by the endolaryngeal microsurgery technique under general anesthesia. A specimen for histologic diagnosis was taken first by means of specially designed scissors with rectangular blades. The pathologic tissue on the upper surface of the vocal fold was easily removed with the use of these scissors. The basement membrane of the epithelium was included in the specimen in order to obtain an accurate histologic diagnosis. The remaining lesion was vaporized with the use of a CO2 laser. Laser vaporization was discontinued when the superficial layer of the lamina propria of the mucosa was removed and the vocal ligament was exposed. The spot size of the laser employed was 800 μm. The lased wound was irrigated with saline solution. The details of the surgical technique have been described elsewhere.[4]

Histopathologically, the lesions were classified

TABLE 1. DISTRIBUTION OF 88 PATIENTS BY BRINKMAN INDEX AND SEX

Brinkman Index	*M*	*F*	*Total*
0	11	7	18
1 - 200	3	1	4
201 - 400	8	0	8
401 - 600	9	0	9*
601 - 800	15	0	15*
801 - 1,000	10	0	10*
1,001 - 1,200	11	0	11*
≥1,201	13	0	13*
Total	80	8	88

*Total of group marked thus is 58 or 66%.

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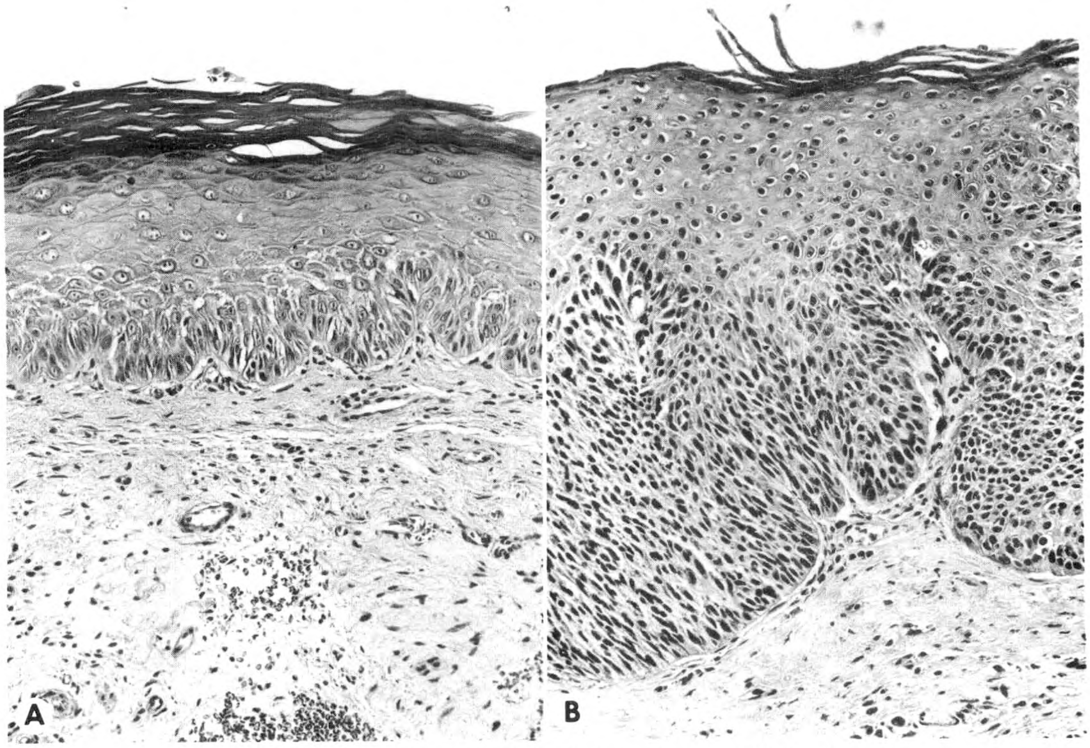

Fig 1. Histologic picture of A) simple hyperplasia and B) dysplasia.

into two groups: simple hyperplasia and dysplasia. The former had no atypia of cells, whereas the latter was associated with cellular atypia (Fig 1). The degree of dysplasia was not graded in this study. Carcinoma in situ and early invasive carcinoma were not included in the present series.

Postoperative follow-up ranged from 12 to 156 months with a mean of 62 months. No systematic voice therapy was given postoperatively.

Vocal function was evaluated before and after surgery in 48 cases. The remaining 40 patients did not receive postoperative evaluation. The parameters employed were 1) maximum phonation time (MPT), 2) mean airflow rate during phonation over comfortable duration (MFRc), 3) fundamental frequency (F0) range of phonation, 4) sound pressure level (SPL) range of phonation, 5) videostroboscopic parameters, including vibratory amplitude and mucosal wave, 6) pitch perturbation quotient (PPQ), 7) amplitude perturbation quotient (APQ), 8) normalized noise energy for 0 to 4 kHz (NNEa), and 9) normalized noise energy for 1 to 4 kHz (NNEb).[6-8] The first two parameters reflect the degree of glottic incompetence, the next two roughly indicate the ability of F0 and SPL control, the fifth parameter reflects the pliability of the vocal fold mucosa, and the last four parameters show some important acoustic aspects of the voice quality. The four acoustic parameters were employed recently, ie, since 1987.

RESULTS OF TREATMENT

Of the 88 patients, 73 were free of recurrence. Sixty-six of the 73 patients were alive, whereas 7 died of intercurrent diseases: 2 died of liver cancer, 1 gallbladder cancer, 1 leukemia, 2 cerebrovascular accident, and 1 myocardial infarction. The other 15 patients developed recurrent lesions. The recurrent lesions were benign in 8 patients and malignant in 7.

Figure 2 depicts the nonrecurrence rate determined by means of the Kaplan-Meier method. The nonrecurrence rate for all the cases was 83% for 5 years and 69% for 10 years (Fig 2A). The nonrecurrence rate was higher for the preoperative nonsmokers than for the smokers (Fig 2B). None of the preoperative nonsmokers developed recurrences. Among the preoperative smokers, postoperative discontinuation of smoking did not affect the nonrecurrence rate (Fig 2C). All patients who developed recurrences were men. The nonrecurrence rate did not differ between simple hyperplasia and dysplasia up to the 5th postoperative year, but it was higher for simple hyperplasia than for dysplasia at the 10th postoperative year (Fig 2D).

Table 2 shows the incidence of development of invasive carcinoma by cigarette smoking and by histologic type. None of the preoperative nonsmokers developed carcinoma. Among the preoperative smokers, the rate of cancer development did not differ between those who discontinued smoking after laser surgery and those who continued smoking. The

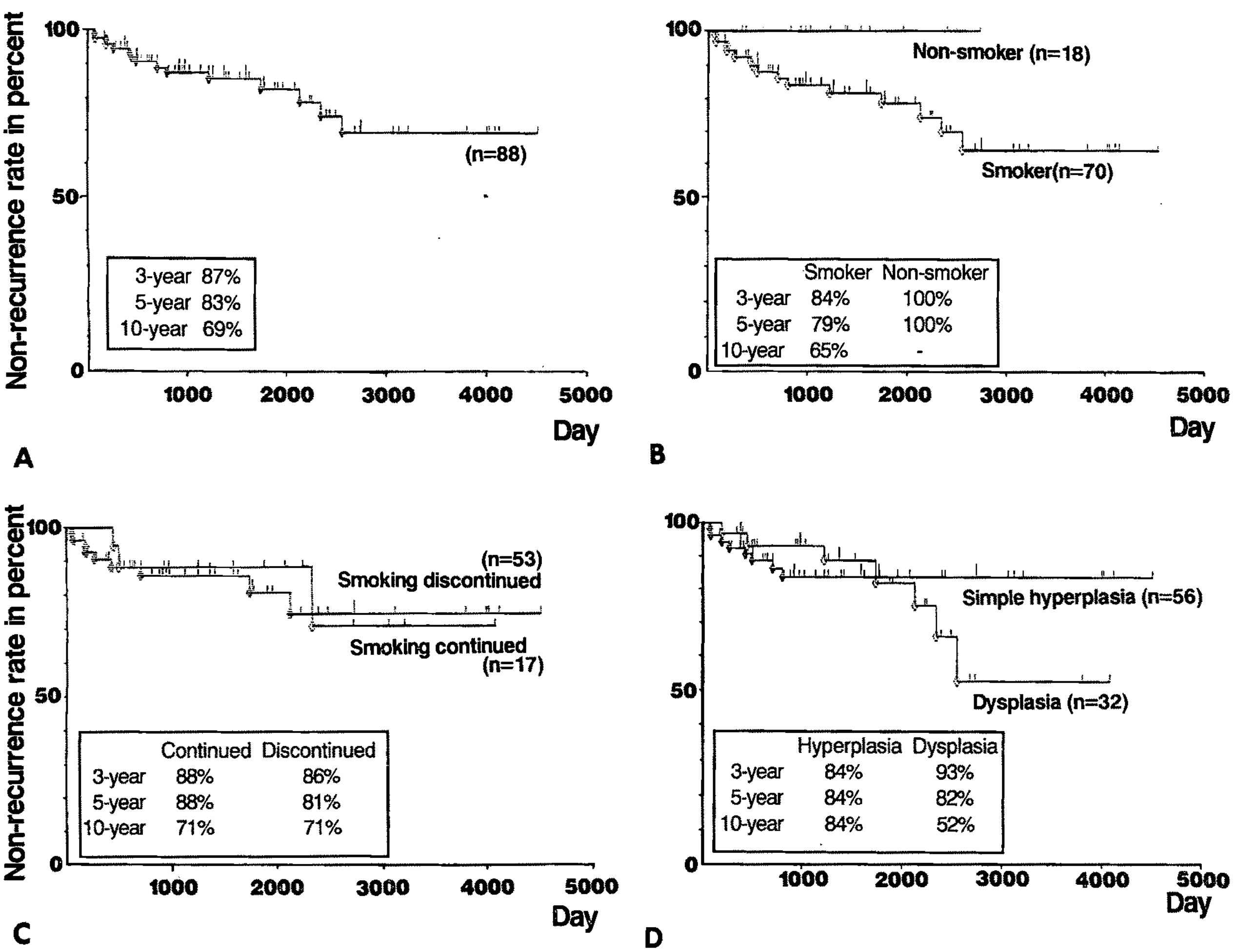

Fig 2. Nonrecurrence rate determined by means of Kaplan-Meier method. A) For all cases. B) For preoperative smokers versus nonsmokers. C) For those who continued smoking postoperatively versus those who discontinued. D) For simple hyperplasia versus dysplasia.

rate of the occurrence of cancer did not differ between simple hyperplasia and dysplasia, either.

Figure 3 depicts an outline of the 15 patients who developed recurrences. Six patients (B1 through B5 and B8) had one recurrence of EH without malignancy, and 2 patients (B6 and B7) developed recurrences of EH twice. The interval between the first laser surgery and the recurrence of EH ranged from 2 to 84 months with a mean of 35 months. All recurrences were treated with laser surgery again. For the 7 patients who developed carcinoma (M1 through M7), the duration between the first laser operation and the development of carcinoma ranged from 6 to 80 months with a mean of 34 months. One patient (M3) had three recurrences of EH before the occurrence of carcinoma, and another patient (M6) had one recurrence of EH prior to the development of carcinoma. The carcinomas were successfully treated by means of radiotherapy in all 7 patients.

VOCAL FUNCTION

In the majority of the patients, the MPT and MFRc value were within normal range before surgery. Following laser surgery, MPT increased and MFRc decreased in many patients (Fig 4A,B). The increase in MPT and decrease in MFRc were more frequent for unilateral lesions than for bilateral lesions. Preoperatively, the F0 range was below the lower border of normal in approximately half the patients, whereas the SPL range was within the normal range in most cases. The F0 and SPL range of phonation did not consistently change postoperatively (Fig 4C,D).

Preoperative and postoperative videostroboscopic findings are shown in Table 3. Videostroboscopic findings were available for 53 affected vocal folds. The vibratory amplitude was normal in 28% and the mucosal wave was normal only in 8% before laser surgery. Following surgery, the amplitude was normal in 45% and the mucosal wave was normal in 43%. Postoperatively, the amplitude of vibration increased in 19, was unchanged in 25, and decreased in 9 vocal folds. The mucosal wave increased in 26, was unchanged in 20, and decreased in 7 vocal folds following surgery.

TABLE 2. INCIDENCE OF CANCER DEVELOPMENT

		Cancer	
	n	*No.*	*%*
Preoperative nonsmoker	18	0	
Preoperative smoker			
Discontinued postoperatively	53	5	9
Continued postoperatively	17	2	12
Simple hyperplasia	56	5	9
Dysplasia	32	2	6

Among the acoustic parameters, PPQ and APQ were within the normal range preoperatively in the majority of cases (Fig 4E,F). The NNEa value was near or below the range of normal in most patients, whereas the NNEb was greater than the range of normal in the majority of the patients before surgery (Fig 4G,H). The acoustic parameters did not show consistent changes following laser surgery (Fig 4E-H).

DISCUSSION

Since 1972, when Strong and Jako[9] reported the use of the CO2 laser in treating vocal fold disorders, the CO2 laser has been employed by many laryngologists for the treatment of EH of the vocal fold. Reports on long-term results and functional results, however, have been rather limited.

The rate of nonrecurrence (recurrences included both benign and malignant lesions) following traditional surgery was reported to be 19 of 24 (79%) by Shin et al,[10] 11 of 14 (79%) by Hellquist and Olofsson,[11] and 91 of 137 (66%) by Højslet et al.[12] In the present study, the nonrecurrence rate determined by the Kaplan-Meier method was 83% for 5 years and 69% for 10 years. Since the follow-up duration and the method to determine the rate of nonrecurrence differ among the different reports, one cannot compare the results between the CO2 laser and traditional techniques. It appears, however, that the use of the CO2 laser does not significantly reduce the recurrence rate. The major advantages of the laser over the traditional technique are, therefore, a greater precision in removing the tissue and much less bleeding during the operation.

In the present series, recurrences were more frequent for preoperative smokers than nonsmokers. Discontinuation of smoking after surgery did not cause any decrease of recurrence.

Development of invasive carcinoma is a major concern for the patients with EH of the vocal fold. Table 4[11-15] shows the incidence of the occurrence of carcinoma reported in some of the previous literature. The incidence of carcinoma development depended on the degree of dysplasia, ie, atypia of epithelial cells, in these reports. In the present series, however, the incidence of carcinoma development did not differ between simple hyperplasia and dysplasia. It was less than 10% for both histologic types, as shown in Table 2. The incidence of occurrence of carcinoma for dysplasia in the present investigation appears to be lower than that shown in Table 4. In the reports listed in Table 4, the CO2 laser was not used in the

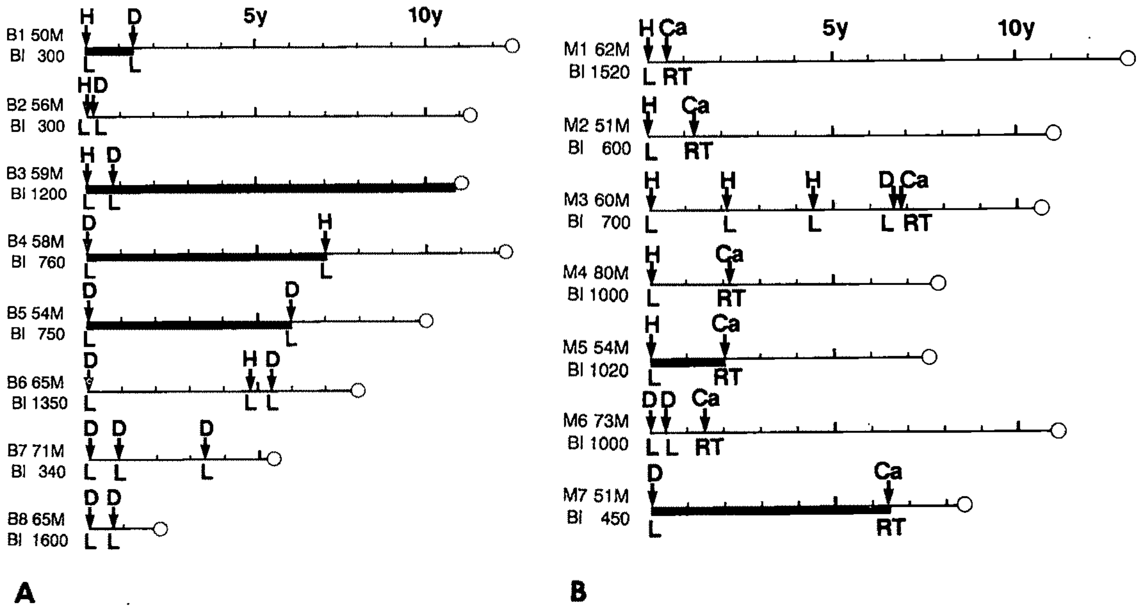

Fig 3. Outline of patients who developed recurrences: A) cases B1 through B8 (benign lesions) and B) cases M1 through M7 (malignant lesions). Numbers to right of case number — age at first laser operation, M — male, BI — Brinkman index, H — simple hyperplasia, D — dysplasia, Ca — carcinoma, open circles — alive with no evidence of disease, thick horizontal line — duration of cigarette smoking following laser surgery, L — laser, RT — radiotherapy.

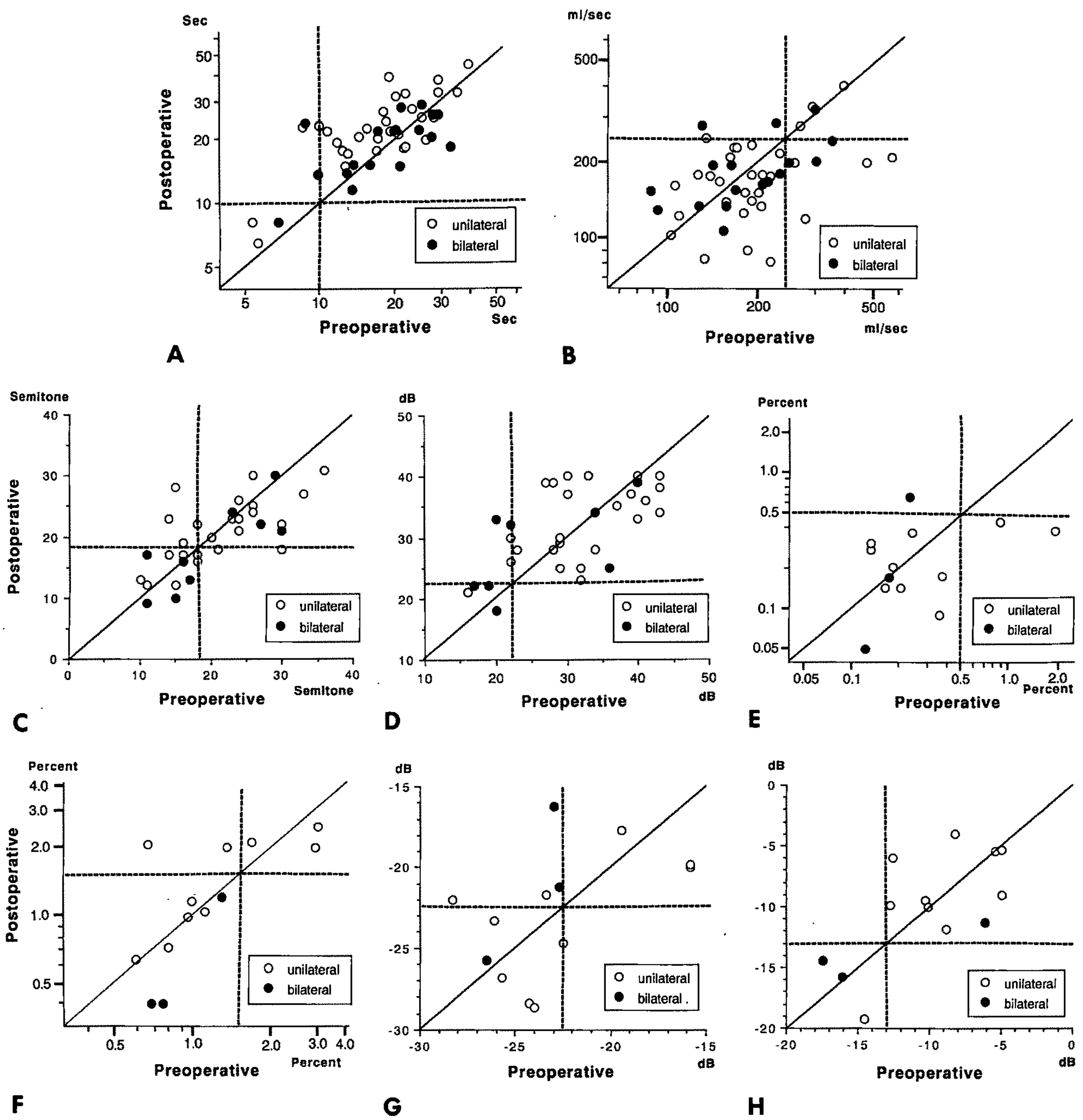

Fig 4. Preoperative and postoperative vocal function. Dashed lines depict border of normal range. Circles above oblique line indicate postoperative increase, and those below oblique line indicate decrease. A) Maximum phonation time. B) Mean airflow rate. C) Fundamental frequency range of phonation. D) Sound pressure level range of phonation. E) Pitch perturbation quotient. F) Amplitude perturbation quotient. G) Normalized noise energy for 0 to 4 kHz. H) Normalized noise energy for 1 to 4 kHz.

treatment, except for a small part of the series by Hirade et al,[14] whereas all patients in the present series were treated with the CO2 laser. This may indicate that the use of the CO2 laser in treating epithelial dysplasia of the vocal fold might inhibit the development of carcinoma to a certain extent. A final conclusion awaits further investigation.

Reports on the vocal function following laser surgery for EH of the vocal fold have been limited. In the present study, MPT and MFRc, ie, the parameters reflecting glottic incompetence, were within the normal range in the majority of the patients with EH, and the laser surgery brought some improvement in many cases. The improvement was more frequent for unilateral lesions than bilateral lesions. This indicates that laser vaporization of the superficial layer of the lamina propria of both vocal folds is more disadvantageous for glottic closure during vocal fold vibration than that of only one vocal fold. The F0 range was more frequently decreased by the existence of EH

TABLE 3. PREOPERATIVE AND POSTOPERATIVE VIDEOSTROBOSCOPIC FINDINGS FOR 53 VOCAL FOLDS

	Preoperatively			
Postoperatively	*Normal*	*Small*	*Zero*	*Total*
Amplitude of vibration (n = 53)				
Normal	7	16	1	24 (45%)
Small	8	18	2	28 (53%)
Zero	0	1	0	1 (2%)
Total	15 (28%)	35 (66%)	3 (6%)	53
Mucosal wave (n = 53)				
Normal	3	12	8	23 (43%)
Small	1	11	6	18 (34%)
Zero	0	6	6	12 (23%)
Total	4 (8%)	29 (55%)	20 (38%)	53

than was the SPL range of phonation. The F0 and SPL range of phonation did not change consistently following laser surgery, indicating that the ability to control the F0 and SPL of phonation was not basically changed following laser surgery.

As for the vibratory behavior of vocal folds with EH, a decrease in amplitude vibrations and mucosal wave has been reported.[16] This was also the case with the present series. Following laser surgery, the amplitude and mucosal wave tended to increase, indicating that the vocal fold tissue was apt to become more pliable following laser vaporization. In the present series, the extent of laser vaporization was apt to be a bit too much. We could have minimized the extent of removal of the superficial layer of the lamina propria in most cases, provided that the depth of the lesion could be somehow predetermined. Stroboscopy seems to be a modality to estimate the depth of pathologic changes.[16] A precise estimation of the extent of the lesion associated with a minimum tissue removal will result in better postoperative vibratory behavior accompanied by better vocal function.

Among the four acoustic parameters, NNEb, which indicates the relative noise level in the high-frequency range, was abnormally increased in the patients with EH of the vocal fold. Neither PPQ nor APQ, which reflect the aperiodicity in vocal fold vibration, was markedly affected by the existence of EH.

The four acoustic parameters did not differ consistently between preoperative and postoperative conditions. In other words, the voice quality for the vocal folds with EH and that for the vocal folds after laser vaporization of Reinke's space were not significantly different, at least according to the acoustic parameters employed in this study.

TABLE 4. INCIDENCE OF DEVELOPMENT OF INVASIVE CARCINOMA REPORTED IN LITERATURE

			Invasive Carcinoma		
Authors	*Histologic Type*	*n*	*No.*	*%*	*Follow-up (mo)*
Hellquist and Olofsson[11]	Hyperplasia and moderate dysplasia	14	0	0	12-166
Hellquist et al[13]	Severe dysplasia and carcinoma in situ	20	5	25	26-151
Hirade et al[14]	Simple hyperplasia	34	1	3	6-120
	Dysplasia	10	3	30	
Kalter et al[15]	Dysplasia	18	10	56	6-132
Højslet et al[12]	Hyperplasia with or without mild dysplasia	128	6	5	11-128
	Moderate dysplasia	9	4	44	
	Severe dysplasia	10	4	40	

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FIRST CONGRESS OF EUROPEAN SKULL BASE SOCIETY

The European Skull Base Society will hold its first congress September 26-30, 1993, in Riva del Garda (Trento), Italy. For further information contact Carmelo Zappone, MD, Congress Secretary, Division of ORL, Ospedali Riuniti, Largo Barozzi 1, 24100 Bergamo, Italy; telephone 35-269578; fax 35-269460.

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CORRELATION BETWEEN THE LARYNGEAL BRAIN STEM EVOKED RESPONSE AND THE LARYNGEAL CHEMOREFLEX IN THE PORCINE MODEL

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The laryngeal brain stem evoked response (LBR) represents the neural activity involved in laryngeal reflex pathways. The laryngeal chemoreflex (LCR) is a centrally mediated response consisting of apnea and hemodynamic changes that result from laryngeal stimulation. The purpose of this study is to determine the characteristics of the LBR that are predictive of LCR severity in the porcine model. The duration of apnea resulting from stimulation of the supraglottic larynx defined LCR severity. The LBR tracings were recorded from electrodes flanking the brain stem following direct electrical stimulation of the superior laryngeal nerve. The LBR peak latencies from piglets demonstrating prolonged LCR apnea were compared to those without an exaggerated LCR response. Two LBR peak latencies demonstrated a statistically significant difference between the two piglet groups. These peak latencies appear to be indicators of susceptibility to exaggerated laryngeal reflex sensitivity. Thus, the LBR may prove useful in identifying and evaluating subjects predisposed to conditions associated with dysfunctional laryngeal reflex activity.

KEY WORDS — brain stem, chemoreflex, evoked potentials, larynx, sudden infant death syndrome, superior laryngeal nerve.

INTRODUCTION

Laryngeal reflexes play an important role in respiratory control and pulmonary protection from aspiration. Mechanical or chemical stimulation of the supraglottic mucosa or direct electrical stimulation of the superior laryngeal nerve (SLN) may result in either or both of two responses (Fig 1[1]). The laryngeal adductor response causes glottic closure.[2,3] The second response, the laryngeal chemoreflex (LCR), results in centrally mediated apnea, hemodynamic instability, and swallowing.[4] There is evidence that pathologic laryngeal reflexes are responsible for several disorders, including idiopathic laryngospasm, spasmodic dysphonia, "reflex" apnea, apnea of infancy, and sudden infant death syndrome (SIDS).[5-7]

Susceptibility to the profound effects of the LCR is related to the age of the animal.[8,9] Stimulation of the supraglottic area or direct electrical stimulation of the SLN during a critical period of postnatal development has been shown to result in fatal apnea. In the porcine model, apnea is most pronounced in the 4- to 29-day age range, and fatal apnea is confined to piglets less than 3 weeks old. Similar age-related changes in responsiveness to laryngeal stimulation have been found in several animal models, including the calf, dog, cat, and monkey.[10-13] Data have been collected in premature human infants that suggest the existence of an LCR response.[14] Thus, it seems that a critical yet transient period of postnatal development exists during which laryngeal stimulation may result in fatal apnea in susceptible neonatal animals.

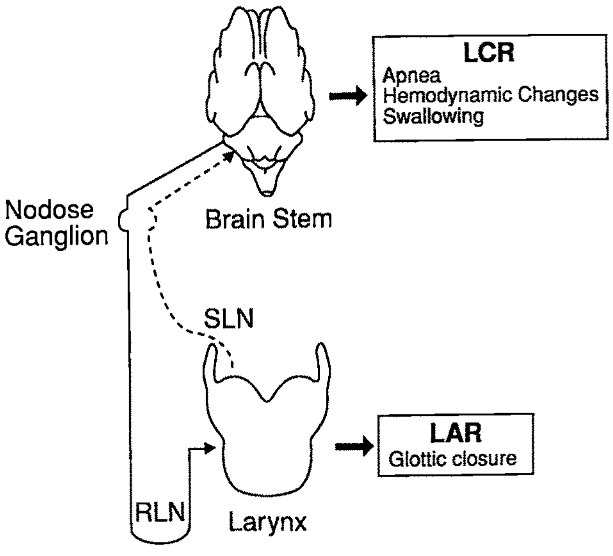

Fig 1. Diagrammatic representation of laryngeal reflexes resulting from supraglottic or direct superior laryngeal nerve (SLN) stimulation. RLN — recurrent laryngeal nerve, LCR — laryngeal chemoreflex, LAR — laryngeal adductor response. (Reprinted with permission.[1])

From the Department of Otolaryngology–Head and Neck Surgery, University of Michigan, Ann Arbor, Michigan. Supported by the Percy Memorial Research Grant of the American Academy of Otolaryngology–Head and Neck Surgery Foundation, Inc. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 11-12, 1992.

REPRINTS — Ramon M. Esclamado, MD, Dept of Otolaryngology, 1500 E Medical Center Dr, Ann Arbor, MI 48109-0312.

TABLE 1. LARYNGEAL BRAIN STEM EVOKED RESPONSE PEAKS IN MATURE PIGLETS

Wave	Latency Range (ms)	Mean Latency (ms)	SD	% Occurrence	Coefficient of Variation	N
P1	0.93-1.61	1.24	0.19	71	0.16	44
N1	1.52-2.10	1.82	0.13	85	0.07	53
P2	2.20-2.69	2.52	0.16	15	0.06	9
N2	2.98-3.18	3.05	0.10	11	0.03	7
P3	2.73-4.09	3.37	0.36	65	0.11	40
N3	3.98-5.23	4.71	0.38	18	0.08	11
P4	4.45-5.60	4.97	0.54	6	0.11	4
N4	5.13-6.20	5.47	0.37	13	0.07	8
P5	5.90-6.50	6.25	0.23	27	0.04	17
N5	6.09-7.38	6.86	0.29	27	0.04	17
P6	6.69-8.06	7.16	0.49	15	0.07	9
N6	7.67-8.16	7.89	0.22	6	0.03	4

Modified from Cohen et al.[1]

This mechanism of fatal apnea is one of the putative causes of SIDS.

In the piglet, age-related changes in laryngeal reflexes do not appear to result from variations in either the peripheral receptor or the myelinization of the SLN.[15] Instead, maturational changes involving the brain stem nuclei controlling respiration are believed to be responsible for the age dependence of the LCR. Anatomic studies of the SLN have demonstrated maturational postnatal variations in its projections to respiratory center nuclei that parallel changes in the apneic and cardiovascular response of the LCR.[9] These brain stem nuclei may play an integral role in mediating pathologic laryngeal reflexes, and an objective measurement of the brain stem activity following SLN stimulation may provide valuable insights into the pathophysiology of laryngeal reflex dysfunction.

In 1987, Isogai et al[16] described a method to elicit averaged electrical activity evoked by SLN stimulation from the brain stem using a far-field recording technique in adult cats. They designated these recordings the laryngeal brain stem evoked response (LBR). The signal to noise ratio is improved by signal averaging, similar to auditory brain stem evoked response testing. The response signal component, which is synchronized to the stimulus, can be amplified and extracted from the noise component. If the neuroelectrical activity of the nuclei evoked by the laryngeal reflex is organized and synchronized to the excitation of the SLN, the brain stem response can be recorded. Isogai et al recorded four positive and four negative peaks in the feline LBR, with latencies ranging from 0.5 to 5.5 milliseconds. Anonsen et al[17] and Lalakea et al[18] further defined the LBR in adult and infant cats.

The porcine LBR was initially described in mature piglets (30 to 45 days of age) in our laboratory.[1] The normative data in these piglets are presented in Table 1.[1] These results were obtained in an identical manner to that used in this paper, employing a recording montage similar to that used by Anonsen et al.[17] Although there was considerable variability in some of the peak latencies, six positive and six negative waves were identified, with a latency range from 1.2 to 7.9 milliseconds. The most reproducible components were P1, N1, and P3. Even though these previous studies have helped define the LBR response, the physiologic correlates of the LBR have not been addressed.

The purpose of this study was to measure the magnitude of the LCR response and record the LBR in neonatal piglets. A comparison of LCR and LBR responses was made to determine whether LBR tracings correlated with LCR severity. Maturational changes in the brain stem nuclei responsible for differential LCR responses should be manifested as variations in LBR latencies. Therefore, LBR peak latencies in piglets susceptible to prolonged LCR apnea and piglets that do not demonstrate an exaggerated response were compared. The LBR, if shown to be a reliable indicator of laryngeal reflex sensitivity, may then serve as a mechanism for identifying and evaluating subjects predisposed to pathologic laryngeal reflexes.

METHODS

Experimental Setup. Mixed breed piglets, 7 to 14 days of age (1.5 to 4 kg) and 30 to 45 days of age (7 to 12 kg), were premedicated with intramuscular Telazol (6 mg/kg), Rompun (3 mg/kg), and atropine sulfate (0.04 mg/kg). Anesthesia was maintained with intramuscular ketamine hydrochloride (10 mg/kg) every 30 minutes. A heating pad, femoral arterial and venous catheters, and a rectal thermometer were placed. Periodically, blood was taken from the arterial line to measure the PO_2, PCO_2, and oxygen saturation. The larynx and trachea were exposed through a vertical midline cervical incision. The

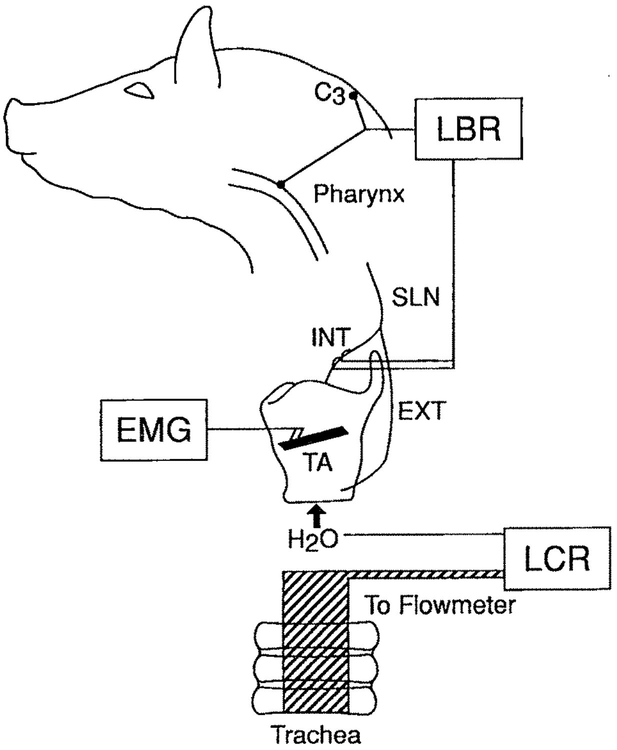

Fig 2. General animal preparation. Laryngeal chemoreflex (LCR) was elicited by supraglottic stimulation with water. Laryngeal brain stem evoked response (LBR) was recorded from pharynx-cervical (C3) montage after stimulation of superior laryngeal nerve (SLN). Thyroarytenoid (TA) muscle electromyographic response (EMG) was also obtained. INT — internal, EXT — external. (Modified from Cohen et al.[1])

trachea was entered below the second ring. A cuffed endotracheal tube was inserted into the distal trachea and connected to a Sechrist model 400 airway pressure monitor to detect respiratory effort. The blood pressure, heart rate, and respiratory rate were recorded with a Hewlett Packard model 7754B chart recorder.

Laryngeal Chemoreflex Determination. The LCR responses were elicited in two age groups of piglets: 30 to 45 days of age and 7 to 14 days of age. These age groups were chosen because piglets in the younger age range are at risk for prolonged LCR-induced apnea, while the older piglets are not. The endotracheal tube cuff was inflated to create a closed circuit between the lungs and the airway pressure monitor. A syringe was inserted through the glottis into the supraglottic region, and 5 mL of distilled water was delivered to the supraglottic mucosa. The syringe tip sealed off the subglottis, restricting the water to the supraglottic area. This stimulation elicited the LCR (Fig 2[1]). The duration of apnea was measured after each injection. Stimulations were performed under both normoxic (arterial oxygen saturation greater than 90%) and hypoxic (arterial oxygen saturation less than 70%) conditions. The distilled water was suctioned from the supraglottic region before each stimulation. The piglets were allowed to return to a baseline physiologic state between stimulations. The mean from the three stimulations provided the LCR apnea duration in each piglet. A paired *t* test was used to determine if a significant difference in apnea duration existed between normoxic and hypoxic stimulations. After the LCR data were collected each piglet underwent LBR measurement.

Laryngeal Brain Stem Evoked Response Measurement. Previous LBR testing had been performed in older piglets to define the adult response (Table 1). Therefore, only piglets 7 to 14 days of age underwent LBR testing. Identical methods that had been used effectively in the prior piglet LBR study were employed. The left SLN was isolated and the thyrohyoid membrane was divided. Grass type E2 platinum subdermal needle electrodes were used to record the brain stem activity and electromyography (EMG) response (Fig 2). The noninverting (active) electrode (P) was placed into the posterior pharyngeal wall. The reference electrode (C3) was secured in the subcutaneous tissue of the posterior neck at the level of the third cervical vertebra. This electrode pair formed a pharynx-C3 (P-C3) recording montage adjacent to the caudal brain stem. Two electrodes were inserted into the left thyroarytenoid muscle for EMG recordings. A ground electrode was placed in the left lower extremity. Two Teflon-insulated platinum-iridium wires (Medwire 3T) were hooked around the internal branch of the left SLN to provide bipolar stimulation to the afferent limb of the reflex arc. A Bio-Logic Navigator Electrodiagnostic Testing System was used to deliver the electrical stimulus to the SLN and to record both the brain stem response from the P-C3 electrode montage and the thyroarytenoid EMG following stimulation.

In the unparalyzed state, evoked compound motor potentials from the thyroarytenoid muscle were used to demonstrate an intact laryngeal reflex arc in each piglet. This EMG response served as a positive control by documenting the adequacy of the SLN stimulus to activate laryngeal reflex efferents. These recordings were also used to determine the completeness of muscular paralysis. Monopolar rectangular pulses (0.1 millisecond) of alternating polarity were delivered at a rate of 11/s. The constant current stimulation intensity was varied from 0 to 12 mA, and each averaged LBR response was derived from 1,500 sweeps. The activity recorded by the P-C3 montage was amplified 100,000 times. The compound motor potentials from the thyroarytenoid muscle were amplified 500 times. Both responses were filtered with a band pass of 10 to 1,500 Hz. A relatively low high-pass filter setting was selected to avoid the ringing

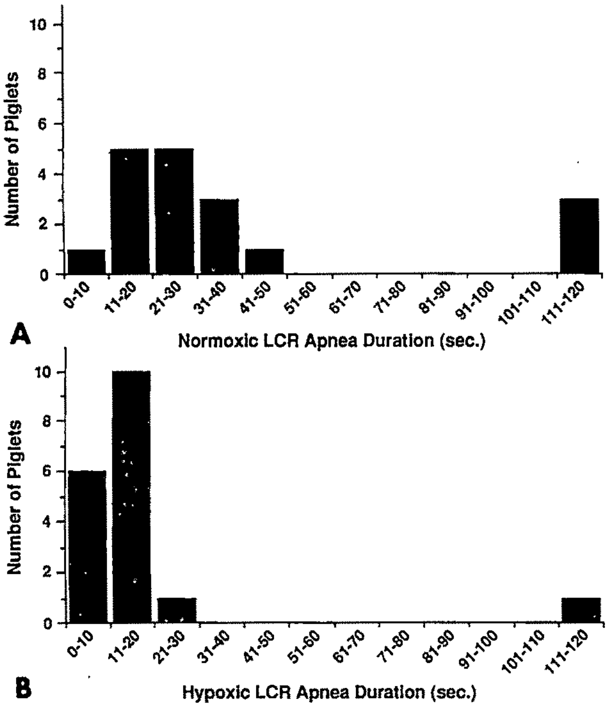

Fig 3. Laryngeal chemoreflex (LCR) apnea duration in neonatal piglets. A) Normoxic conditions. B) Hypoxic conditions.

artifact derived from the electrical stimulus.

Initial LBR recordings were attempted while each piglet was spontaneously breathing. This stimulation resulted in complete apnea in every piglet, requiring resuscitation with artificial ventilation. Recordings, therefore, could not be completed in the spontaneously breathing state. The piglets were then completely paralyzed with intravenous pancuronium bromide (0.3 mg/kg) and mechanically ventilated with a Harvard respiration pump (model 607). All recordings were obtained in the paralyzed condition. After LBR data collection, the SLN was transected proximal to the stimulating electrodes, and the stimulation was repeated. This provided a negative control and served to isolate the stimulus artifact.

The LBR consisted of positive (P1, P2, etc) and negative (N1, N2, etc) peaks following the stimulus artifact (P0, N0). Only those peaks that were unanimously judged significant by three of the authors who independently analyzed the tracings were considered. A series of consecutive latency intervals were defined. In order to be consistent with peak numbering and allow for meaningful intersubject comparisons, waves were labeled according to the latency interval in which they occurred. For example, positive peaks occurring in the 2.7 to 4.7 millisecond range were designated as P3. Simple linear regression analysis was used to evaluate the effect of varying the stimulus intensity on LBR peak latencies.

To determine the most consistent LBR peak latencies, the occurrence rate of each peak and the coefficient of variation for its latency were calculated. The LBR data from piglets susceptible to prolonged LCR apnea were analyzed separately from the data in piglets without an exaggerated LCR response. This enabled a comparison to be made between the LBR findings for these two groups of piglets. Analysis of variance was performed to determine whether there were significant differences in the LBR peak latencies between neonatal piglets demonstrating pro-

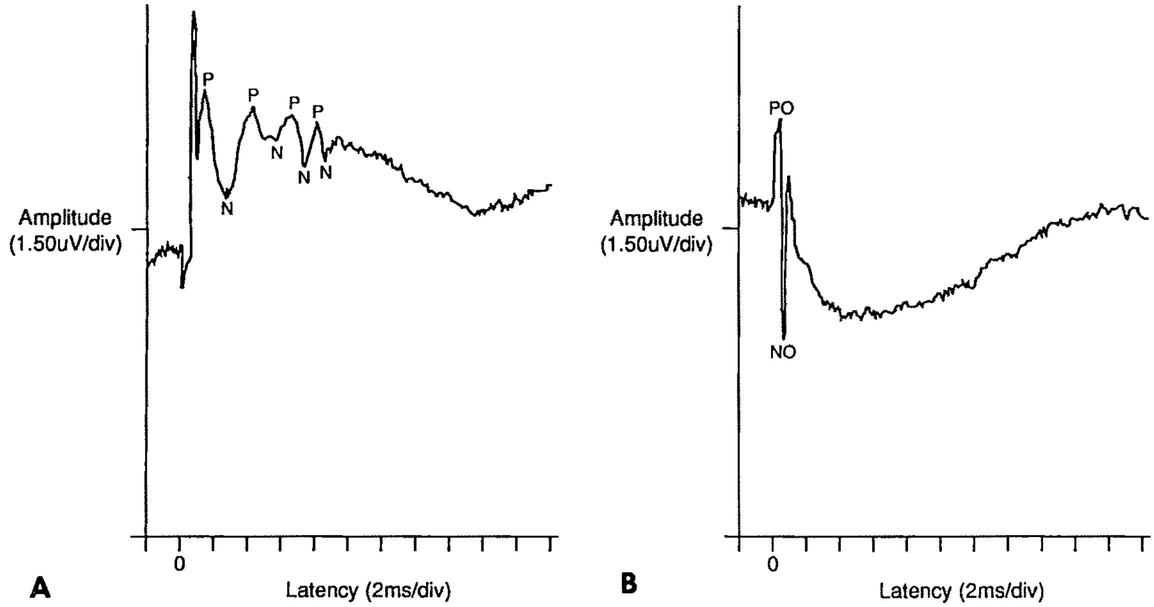

Fig 4. Laryngeal brain stem evoked responses obtained in paralyzed, normoxic, eucapneic state in neonatal piglets. A) Stimulation of intact superior laryngeal nerve (SLN). P — positive peak, N — negative peak. B) Stimulation of SLN after transection. P0, N0 — stimulus artifact.

TABLE 2. LARYNGEAL BRAIN STEM EVOKED RESPONSE PEAKS IN PIGLETS BY GROUP

Wave	*Latency Range (ms)*	*Mean Latency (ms)*	*SD*	*% Occurrence*	*Coefficient of Variation*	*N*
Group A						
P1	0.93-1.42	1.14	0.16	72	0.14	36
N1	1.22-2.20	1.61	0.21	72	0.13	36
P2	1.90-2.49	2.15	0.16	36	0.07	18
N2	2.49-2.98	2.76	0.12	38	0.04	19
P3	3.08-4.74	3.73	0.51	62	0.14	31
N3	3.47-4.93	4.39	0.54	18	0.12	9
P4	5.03-5.42	5.13	0.17	10	0.03	5
N4	5.32-6.20	5.59	0.22	26	0.04	13
P5	6.01-6.50	6.25	0.20	8	0.03	4
N5	6.20-7.08	6.55	0.31	14	0.05	7
P6	6.79-8.25	7.33	0.47	30	0.07	15
N6	7.57-8.94	8.23	0.53	28	0.06	14
Group B						
P1	0.73-1.61	1.16	0.21	70	0.18	41
N1	1.13-2.30	1.64	0.28	70	0.17	41
P2	1.71-2.59	2.26	0.21	61	0.09	36
N2	2.59-3.00	2.80	0.12	53	0.04	31
P3	2.98-4.00	3.40	0.30	44	0.09	26
N3	3.47-4.93	4.50	0.46	44	0.10	26
P4	4.45-5.32	4.98	0.30	27	0.06	16
N4	5.03-5.71	5.38	0.23	24	0.04	14
P5	5.71-6.50	6.15	0.22	54	0.04	32
N5	6.40-7.67	6.94	0.41	34	0.06	20
P6	6.79-8.00	7.33	0.50	20	0.07	12
N6	7.38-8.94	8.28	0.50	37	0.06	22

longed LCR apnea and those with a less pronounced response. Homoscedasticity was assured by Bartlett's test for homogeneity of group variances. The comparison of means with unequal variances was performed with Welch's approximate *t* test. Tukey's procedure was used to control for the effects of multiple comparisons.

RESULTS

The apneic range for piglets 30 to 45 days old stimulated under normoxic conditions was 8 to 15 seconds. The mean for these piglets was 13 seconds. The range and mean for hypoxic stimulations in this group were 6 to 13 seconds and 12 seconds, respectively. In these mature piglets the duration of apnea obtained under normoxic conditions did not differ significantly from that associated with hypoxic stimulations.

The apneic range for piglets 7 to 14 days old stimulated under normoxic conditions was 10 to 120 seconds. The mean and SD were 40 seconds and 36.6, respectively. During hypoxic conditions the apneic range was 1 to 120 seconds, with a mean of 17.9 seconds and an SD of 26.3. This decrease in apnea duration noted in younger piglets under hypoxic conditions, compared to normoxic stimulations, was highly significant ($p = .004$; Fig 3).

The younger piglets (7 to 14 days old) were divided into two distinct groups based on their individual normoxic LCR apnea duration. Group A consisted of piglets with a mean apneic period of less than or equal to 26 seconds. There were 10 piglets in this group. The remaining piglets, group B, all had a mean apnea duration of greater than 26 seconds, which was believed to represent an abnormally prolonged LCR response. There were 9 piglets in this group. The cutoff of 26 seconds was selected because it was the median value for the neonatal piglets and was twice the mean LCR apnea duration for older piglets (30 to 45 days old), who are not susceptible to prolonged apnea.

Only the younger piglets underwent LBR testing. All these piglets demonstrated clear and robust LBR tracings. These waveforms consisted of a series of positive and negative peaks representing laryngeal reflex pathway activation that followed the stimulus artifact, as shown in Fig 4. Also illustrated in this Figure is the effect of sectioning the SLN proximal to the stimulating electrodes. Only the initial peaks (P0, N0) corresponding to the stimulus artifact remain after transection. Table 2 lists the individual peaks identified, along with their absolute latency range, mean latency, and SD. Absolute peak latencies were the primary focus of attention, since it was hypoth-

TABLE 3. LATENCY COMPARISONS

	Present Study		*Near-Field Studies*
Generator Source	*Wave*	*Latency (ms)*	*Latency (ms)*
Ipsilateral vagus nerve	P1	1.1	1.1 ± 0.9[20]
	N1	1.6	
Solitary tract nucleus	P2	2.2	2-4[21-23]
	N2	2.8	
	P3	3.4-3.7	
Nucleus ambiguus	N3	4.4	5-8[22,24]
	P4	5.1	
	N4	5.6	
	P5	6.2	
Reticular formation	N5	6.6-7.0	7[21]
	P6	7.3	
	N6	8.2	

esized that variations in laryngeal reflex sensitivity may correlate with characteristic wave latency changes. Wave amplitudes were too highly variable, even under identical stimulus conditions in the same piglet, for reliable comparisons. Since changes in suprathreshold stimulus intensity produced negligible variation in the peak latencies, all LBR trials were grouped according to physiologic condition for further evaluation.

In an earlier study[1] defining the piglet LBR in this laboratory, it was shown that muscular artifact significantly altered the LBR waveforms. Therefore, for comparison purposes in this study, only LBR tracings obtained under paralyzed conditions were evaluated. A total of 109 LBR tracings were recorded. The waveform illustrated in Fig 4 represents a characteristic tracing obtained in the paralyzed, normoxic, eucapneic state. A total of six positive (P1 through P6) and six negative (N1 through N6) peaks were detected in the LBR waveforms obtained in this physiologic state. However, there was considerable inconsistency in the configuration of some of these peaks.

To determine the intersubject reproducibility of the component LBR waves in the paralyzed, normoxic, eucapneic state, the frequency of peak occurrence and the coefficient of variation for its latency were calculated. These data are presented in Table 2. Fifty tracings were analyzed in the piglets that did not exhibit an exaggerated LCR apneic response (group A). The coefficient of variation for all component peak latencies was less than 0.2; however, only P1, N1, and P3 were present in greater than 40% of the tracings. Fifty-nine tracings from piglets susceptible to prolonged LCR apnea (group B) were evaluated. While all the peak latencies had a coefficient of variation less than 0.2, only waves P1, N1, P2, N2, P3, N3, and P5 occurred in more than 40% of the observations.

When LBR peak latencies from piglets in group A were compared to those in group B, the following results were observed. For most of the peaks there was no significant difference in latency between the two groups of piglets. Only P3 and N5 latencies differed significantly between the two groups: P3 had a mean latency of 3.73 milliseconds with an SD of 0.51 in group A and a mean latency of 3.40 milliseconds with an SD of 0.30 in group B; and N5, with a mean latency of 6.55 milliseconds and an SD of 0.31 in group A, had a mean latency of 6.94 milliseconds with an SD of 0.41 in group B.

DISCUSSION

Laryngeal reflexes provide some control over respiration and protection from aspiration. These functions are vital to the survival of the organism, but under certain circumstances these reflexes can become pathologic and lead to several disorders. A hyperactive laryngeal adductor response can cause spasmodic dysphonia. The LCR, which results in centrally mediated apnea, hemodynamic changes, and swallowing following laryngeal stimulation, may become pathologic in susceptible subjects, resulting in prolonged or fatal apnea.[4,19] There is evidence that an exaggerated LCR response may underlie several disorders, including "reflex" apnea, apnea of infancy, and SIDS.[5-7]

A reliable method of evaluating laryngeal reflex sensitivity could prove useful for studying subjects afflicted with disorders related to laryngeal reflex dysfunction. Currently, no noninvasive recording method has been shown to correlate with laryngeal reflex activity. The LBR, which represents the reflex neural activity following direct electrical stimulation of the SLN, may fill this role. The goal of this study was to determine whether the LBR could serve as an accurate indicator of laryngeal reflex function.

In this study, the degree of apnea resulting from the LCR response served as a barometer of laryngeal reflex sensitivity. The LCR apnea duration was measured during both normoxic and hypoxic conditions. Prior LCR studies revealed a tendency toward prolonged apnea when the response was elicited in various animal models under conditions of reduced oxygen exposure.[8,9] Therefore, hypoxic recordings were obtained in the present study in order to detect a latent exaggerated LCR response that might not have been revealed if only normoxic stimulations had been performed. In contrast to previous reports, the degree of apnea recorded in the neonatal piglets (7 to 14 days old) during hypoxic conditions was significantly less than that observed during normoxic stimulations. This result is consistent with physiologic principles. Hypoxemia normally acts as an impetus to increase respiration. Therefore, it is reasonable to expect that reflex responses resulting in apnea would be blunted during hypoxic conditions. The reasons for the differences observed in this study when compared to prior reports are unclear, but may be related to the method of stimulation or the technique used to quantify the LCR response.

Prior studies have revealed that older piglets have less LCR apnea than younger piglets, with prolonged or fatal apnea restricted to piglets less than 21 days old.[5,8] Within the younger piglet group, there are those that appeared particularly susceptible to prolonged apnea. The findings in the present study were consistent with prior reports. The apnea duration in mature piglets was relatively brief, while neonatal piglets typically displayed a more pronounced apneic response. The LCR apnea duration under normoxic conditions was used to divide these younger piglets into two distinct groups: piglets demonstrating prolonged apnea and those exhibiting a less pronounced response. Since maturational changes involving brain stem nuclei controlling respiration are believed to be responsible for the differences in LCR apnea, it is reasonable to expect that a measurement of this brain stem activity, such as the LBR, might differentiate between these two groups of younger piglets.

In this study, the averaged brain stem activity elicited by SLN stimulation was recorded. The same electrode montage and similar stimulus parameters that had been found effective for recording laryngeal evoked potentials in a prior piglet study were used. The waveforms obtained in this study are believed to represent LBR neural activity initiated by SLN stimulation for the following reasons. First, the initial peak occurred after SLN stimulation in each observation. Consistent responses to stimuli of alternating polarities suggest that these waves represent nonrandom activity triggered by SLN stimulation. The latency of the peaks preceded the thyroarytenoid compound motor potential response, thereby placing them in the appropriate time epoch. Transection of the SLN abolished all the peaks, except those consistent with the stimulus artifact. Finally, that the waveforms persisted despite complete muscular paralysis suggests a neurogenic origin for the peaks.

The LBR tracings consisted of several positive and negative peaks within a 0.1 to 9.0 millisecond latency range. A comparison of the far-field data in this study with prior near-field reports following electrical stimulation of the SLN allows speculation as to the generator sources of the LBR peaks, as shown in Table 3.[20-24] All LBR peaks fell within the expected latency range for neural activity following SLN stimulation, as revealed in the near-field recordings. Nevertheless, the relationships between the LBR peaks and generator sources are merely suggestive, since firm conclusions regarding the anatomic sources of the LBR peaks would require lesion studies or stereotactic recordings from generators along the reflex pathway.

The most frequently occurring LBR peaks were P1, N1, and P3 in group A piglets and P1, N1, P2, N2, P3, N3, and P5 in group B. In both groups there were several other waves present in a smaller percentage of tracings at consistent latencies. Similar observations were demonstrated in an earlier LBR study of older piglets (30 to 45 days), in which only one fourth of the peaks were present in greater than 50% of the tracings, as seen in Table 1. The fact that the less frequently detected waves in younger and older piglets occurred at such characteristic and reproducible latencies suggests that they, like the more frequently occurring peaks, are representative of actual neural activity associated with SLN stimulation, as opposed to artifact.

Although every effort was made to conform to sound electrophysiologic principles, there was considerable variation in interpiglet peak reproducibility. The variability in peak occurrence may be explained in different ways. Some variability may be due to the sensitivity of the recording montage for the individual waves. Placement of the recording electrodes in a different orientation or in closer proximity to the brain stem may obviate the problem of detecting the less frequently observed neural signals and needs further investigation. Another likely explanation for the variability relates to the complexity of the reflex itself. Studies measuring other responses modulated by the reticular formation, such as the vestibulo-ocular reflex, have demonstrated that the state of arousal has a significant impact on these reflexes.[25,26] Since the reticular formation plays a role in laryngeal reflexes, factors that influence reticular formation activity, such as the state of arousal and the level of anesthesia, could affect reflex function. This effect may be reflected in the LBR as variations in either the presence or latency of individual peaks, or both.

The LBR and LCR responses were compared in this study to determine if the LBR could serve as a reliable predictor of laryngeal reflex sensitivity. The peak latencies from piglets with prolonged LCR apnea (group B) and those with a less pronounced response (group A) were contrasted. Although peak N5 showed a difference in latency between the group of piglets, it was present in only 14% of tracings from group A. Only one wave (P3) occurred in a substantial number of tracings and demonstrated a statistically significant difference in latency between the two groups of piglets. The P3 latency was decreased in neonatal piglets demonstrating an exaggerated LCR response. Thus, P3 latency may prove to be a reasonable indicator of laryngeal reflex sensitivity. The data in Table 3 reveal that P3 latency coincides with activity in the solitary tract nucleus. This suggests that there may be a decreased inhibitory effect on the laryngeal reflex pathway in piglets susceptible to an exaggerated LCR apneic response at the level of the solitary tract nucleus, which is reflected as a shortened P3 latency. The possibility exists, however, that this P3 finding is spurious because of multiple comparison problems in an exploratory analysis such as this, and further study would be required to evaluate the significance of this finding.

The LBR may have potential use in human subjects if the following conditions can be satisfied. First, the LBR response must be characterized in humans, preferably employing a relatively noninvasive method. The problems with peak reproducibility seen in this study must be overcome, and a different recording technique may be required. Finally, the human LBR must be shown to be a reliable indicator of laryngeal reflex sensitivity. If these criteria can be met, the LBR could prove clinically useful for identifying and evaluating subjects with disorders related to pathologic laryngeal reflex function.

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HIGH JUGULAR BULB: IMPLICATIONS FOR POSTERIOR FOSSA NEUROTOLOGIC AND CRANIAL BASE SURGERY

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The suboccipital-retrosigmoid approach to the internal auditory canal and cerebellopontine angle is being used with increasing frequency for neurotologic surgery, including vestibular nerve section and resection of acoustic neuroma. It offers wide exposure of the cerebellopontine angle and the cranial nerve VII-VIII complex as it courses from the brain stem to the temporal bone. Exposure of the internal auditory canal can be achieved by removing its posterior bony wall. Safe utilization of this approach requires familiarity with the variable position of structures within the petrous bone, including the lateral venous sinus and jugular bulb. We report here a case in which bleeding resulted from injury to a high jugular bulb during surgical exposure of the internal auditory canal via the suboccipital route and discuss the regional anatomy of the jugular bulb based on study of 378 consecutive temporal bone specimens from the collection of the Massachusetts Eye and Ear Infirmary. High jugular bulb was defined as encroachment of the dome of the bulb within 2 mm of the floor of the internal auditory canal. Forty-six percent of scoreable specimens met this criterion. However, when donors less than 6 years of age were excluded, a high jugular bulb was identified in 63% of specimens. Relevance to neurotologic surgery of the posterior fossa is presented.

KEY WORDS — cranial base surgery, internal auditory canal, jugular bulb, posterior fossa, neurotologic surgery, temporal bone anatomy.

INTRODUCTION

The suboccipital-retrosigmoid approach to the internal auditory canal (IAC) and the cerebellopontine angle is being used with increasing frequency in neurotologic procedures, including section of the vestibular nerve and resection of acoustic neuroma. It offers wide exposure of the cranial nerve VII-VIII complex as it courses from the brain stem to the temporal bone. Exposure of the contents of the IAC is accomplished by removal of the posterior bony canal wall. Safe utilization of this approach requires familiarity with the variable position of structures within the petrous bone, including the lateral venous sinus (LVS) and the jugular bulb (JB).

There have been several reports of a laterally located high JB presenting in the middle ear. The first report of clinical consequences of a high JB was the description by Page[1] in 1914 of hemorrhage during myringotomy. Gejrot[2] reported on radiographic delineation of high JB, including a historical review of 10 cases up to 1942. In a review of congenital ear malformations, Hough[3] added an additional 4 cases of hemorrhage after elevation of a tympanomeatal flap, believed due to a high JB. Other case reports describe the anatomic relationship of a high JB to the middle ear and mastoid.[2,4-6] In a study of 257 cadaver specimens, Overton and Ritter[7] found a high JB, presenting in the middle ear or mastoid, in 13 cases or 5%.

Far less has been written on the proximity of a medially located high JB to the IAC and its importance in posterior fossa surgery. Although Graham[8] has noted the anatomic relationship of the JB and LVS to the IAC and posterior semicircular canal and the potential for bleeding during exposure of the IAC for removal of acoustic neuroma, in a review of the history, anatomy, and surgical management of the JB[9] he makes no further comments regarding the JB in posterior fossa neurotologic surgery. Case reports of a true proximity of a high JB to the inner ear[10] and IAC have appeared.[11,12] In one report a diverticular extension of the medial portion of the JB encroached on the posterior wall of the IAC.[12] The clinical relevance of a high JB and the potential for intraoperative hemorrhage have been described in surgery of the IAC[13,14] and endolymphatic sac.[14] We report here another case in which surgical injury to a high JB via the suboccipital route for removal of an acoustic neuroma resulted in hemorrhage and difficult exposure of the IAC. Our experience with this case prompted us to study the regional anatomy of the JB and its proximity to other structures in 378 consecutive temporal bone specimens from the collection of the Massachusetts Eye and Ear Infirmary.

CASE REPORT

A 39-year-old man had a 2-year history of progressive left unilateral sensorineural hearing loss with

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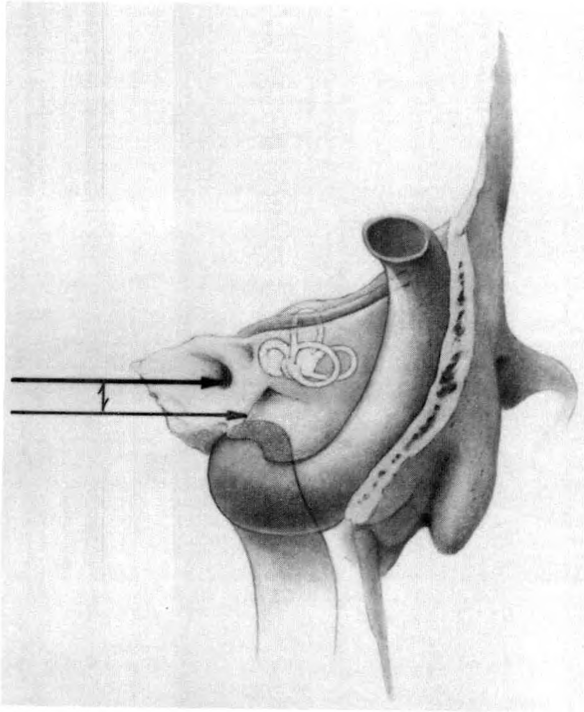

Fig 1. Artist's rendering of posterior view of right temporal bone showing course of sigmoid sinus and jugular bulb. Bold lines denote lower lip of porus acusticus and apex of dome of jugular bulb. High jugular bulb was defined as distance of 2.0 mm or less between these two lines.

reduced speech discrimination, left facial hypesthesia, and mild disequilibrium. Brain stem auditory evoked potentials demonstrated prolonged latency on the left side, consistent with an ipsilateral retrocochlear lesion. Electronystagmography showed a reduced caloric response on the left. A high-resolution computed tomography (CT) scan confirmed a 2.5-cm mass in the left cerebellopontine angle, with widening of the IAC, consistent with an acoustic neurinoma.

At suboccipital craniotomy for removal of the tumor, the cerebellum and brain stem were retracted medially and the medial end of the tumor was dissected free. A high-speed drill was used to open the IAC. Brisk bleeding was encountered at the inferior margin of the osteotomy, requiring Surgicel tamponade and packing, and preventing removal of the lateral extent of the tumor. The procedure was terminated at that point and the craniotomy closed. The patient had an uneventful postoperative course. Subsequent CT scanning in the axial and coronal planes revealed a high, medially located JB immediately adjacent to the medial end of the IAC. Three months later a combined suboccipital and retrolabyrinthine approach was used to skeletonize and compress the LVS and JB to gain access to the entire IAC and complete the removal of tumor. Recovery was uneventful.

RESULTS OF TEMPORAL BONE MEASUREMENTS

	High JB		*Low JB*	
	No.	*%*	*No.*	*%*
Total bones	49	100	58	100
<6 years old	2	4	30	52
≥6 years old	47	96	28	48
Female, R	12	25	10	17
Female, L	15	31	11	19
Male, R	14	29	21	36
Male, L	8	16	16	28
Total female	27	55	21	36
Total male	22	45	37	64
Total R	26	53	31	53
Total L	23	47	27	47

Percentages are relative to total bones with specified configuration (high versus low JB) noted in first row of table.

JB — jugular bulb.

METHOD AND MATERIALS

Temporal Bone Anatomy. The anatomy of the JB was reviewed in 378 consecutive human temporal bones from the collection of the Massachusetts Eye and Ear Infirmary. The specimens were fixed in Heidenhain-Susa solution, decalcified with trichloroacetic acid, embedded in celloidin, and sectioned in the midmodiolar (horizontal) plane at a thickness of 20 μm. Every 10th section was stained with hematoxylin and eosin and mounted. Thus, there was a vertical distance of 200 μm between adjacent slides. The vertical distance between the inferior edge of the internal auditory meatus and the apex of the dome of the JB was calculated and correlated with age, sex, and laterality of the specimen.

A JB was defined as "high" if there was a vertical distance of 2.0 mm or less between the apex of the dome and the inferior lip of the internal acoustic meatus (Fig 1). Specimens that did not meet this 2.0 mm criterion were considered to have a "low" JB. Specimens in which the JB was not visible were measured to determine the vertical distance between the inferior margin of the internal acoustic meatus and the most inferior section of the specimen. Specimens were excluded from the study if the plane of section was vertical, if the sex of the donor was unknown, or if the specimen was inadequate because of a lack of sufficient basal sections or removal artifact.

An additional specimen was cut parallel to the plane of the posterior fossa surface of the temporal bone in order to demonstrate the anatomic proximity of the LVS and JB to the structures of the inner ear and IAC. This plane of section closely approximates the orientation of the surgeon's view obtained during posterior fossa exposure of the IAC.

Three-dimensional Computer-Assisted Recon-

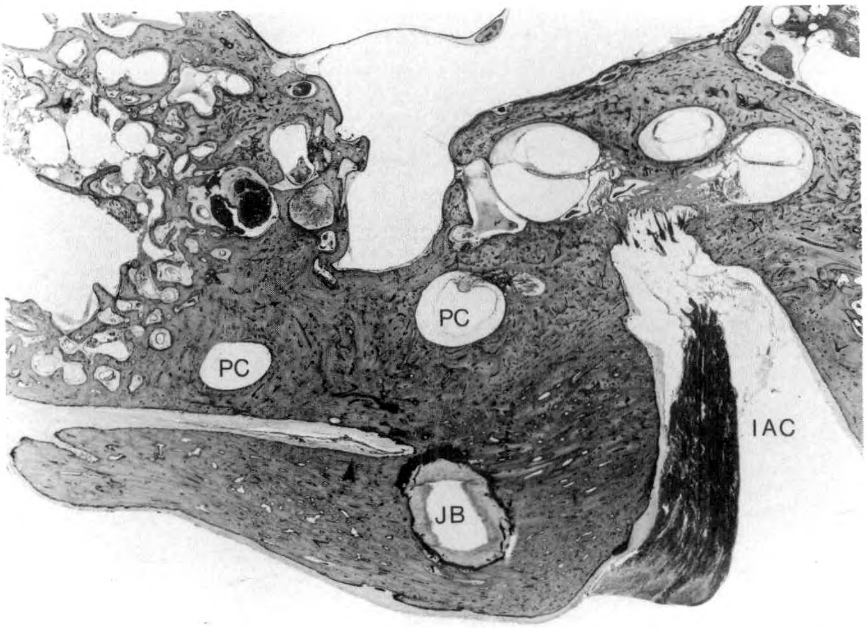

Fig 2. Horizontal section of left human temporal bone showing anatomic relations of jugular bulb (JB) to vestibular aqueduct and endolymphatic sac (original x7.2). Arrowhead — junction of endolymphatic duct and sac, IAC — internal auditory canal, PC — posterior semicircular canal.

struction. Computer-assisted three-dimensional reconstructions were performed on seven cases of high JB. After visual alignment and registration marking of slides, each section of a specimen was projected and boundaries of the IAC, JB, facial nerve, bony labyrinth, endolymphatic duct, ossicles, and, in some specimens, the middle ear were traced. These tracings were then digitized with the Hitachi digitizing tablet HD-111c and a MacIntosh II microcomputer. Three-dimensional reconstructions were created with MacReco (Otten and Dreschel, Groningen, the Netherlands), a software package specifically designed for reconstruction of serially sectioned biologic specimens. Wire-frame reconstructions were displayed as stereo pairs or as animated rotations in order to qualitatively assess the anatomic relations of the digitized structures. Reconstructions were also performed on IAC and JB contours from two CT scans traced in similar fashion. Data files acquired in MacReco were also transferred to MacSpin (D^2 Software, Austin, Tex) to permit color display and real-time rotations in any combination of pitch, roll, and yaw.

RESULTS

Three hundred seventy-eight consecutive temporal bone specimens were analyzed. Two hundred seventy-one were excluded: 16 were sectioned in the vertical plane, 2 were obtained from a donor of unknown sex, 4 were inadequate because of removal artifact or incomplete slide sets, and in 249 specimens

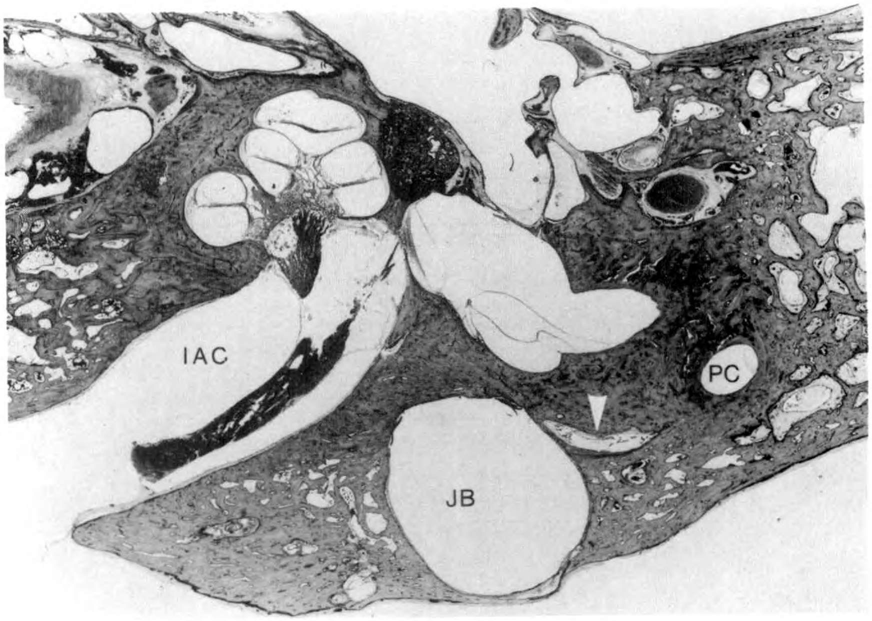

Fig 3. Horizontal section of right human temporal bone showing anatomic relations of jugular bulb (JB) to posterior semicircular canal (PC) and internal auditory canal (IAC; original x7). Arrowhead — vestibular aqueduct.

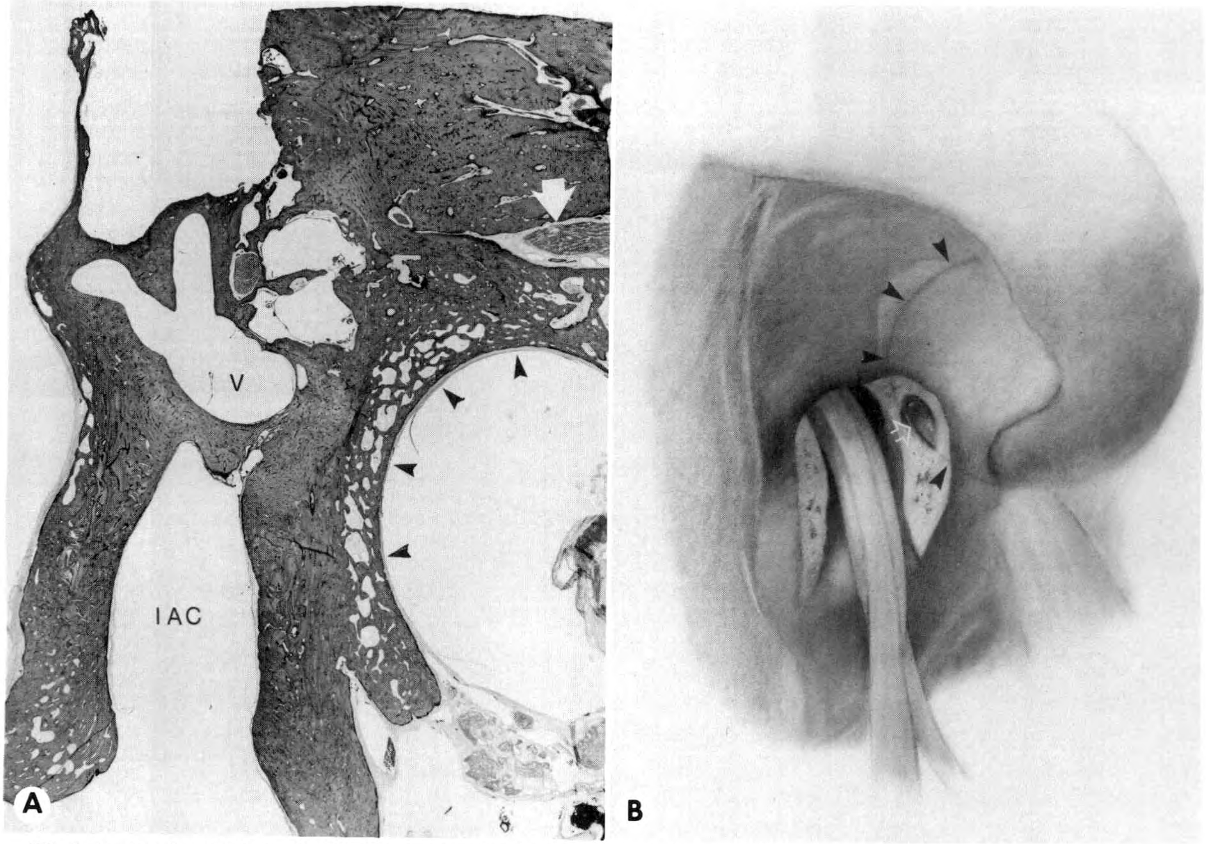

Fig 4. Anatomic relations of critical structures in plane of posterior fossa surface of temporal bone. A) Section of human temporal bone in plane parallel to posterior fossa surface, showing anatomic relations of jugular bulb (arrowheads) to facial nerve and internal auditory canal (IAC; original x5.8). V — vestibule, white arrow — descending facial nerve. B) Artist's rendering of surgical approach to contents of right IAC with patient in left lateral decubitus position. Dome of jugular bulb (arrowheads) is exposed tangentially as bone removal progresses. Open arrow — exposed dome.

less than 2.0 mm of tissue was sectioned below the inferior lip of the internal meatus and no JB was visible in the specimen, precluding determination of the presence of high JB by our definition. The remaining 107 specimens from 81 donors could all be definitively scored by the 2.0 mm criterion, since 50 of the 107 (47%) had a visible JB and 57 of the 107 (53%) had at least 2.0 mm of tissue sectioned below the inferior lip of the internal meatus with no JB in the specimen (see Table).

Forty-nine of the 107 (46%) specimens that could be definitively scored had a high JB by the 2.0 mm criterion, and 58 of the 107 (54%) did not. Within the high-JB group there was approximately equal sex and laterality distribution. Two of the 49 (4%) high-JB temporal bones were from infants or children less than 6 years of age. The incidence of high JB among children less than 6 years of age was 2 of 32 or 6%, compared to 47 of 75 or 63% for older individuals ($p < .001$). Bilateral high JB was noted in 6 cases.

In the low-JB group the distribution of right and left ears was approximately equal. This group had a male predominance of 37 of 58 (64%) male versus 21 of 58 (36%) female. Thirty of 58 (52%) low-JB temporal bones were from young children, with equal sex distribution.

High JBs were often observed to overlap the endolymphatic sac, and sometimes the endolymphatic duct (Fig 2). However, we observed only one case with bony dehiscence between the JB and the endolymphatic sac. When the dome of the JB is high, it may be in close relation to the IAC and posterior semicircular canal (Fig 3). We observed no cases of high JB dehiscent into the IAC. If the dome of the JB is lower, it relates more closely to the hypotympanum, the descending segment of the facial nerve, and the external auditory canal (Fig 4A). The lateral wall of particularly broad JB domes may be in close proximity to the descending facial nerve, even if the apex of the dome is high.

Because of the plane of section, it was impossible to obtain a reliable measure of the medial-to-lateral position of the high JB relative to the other structures of the inner ear, since several intervening sections were present between the inferior lip of the IAC and the dome of the JB, and no consistent medial or lateral

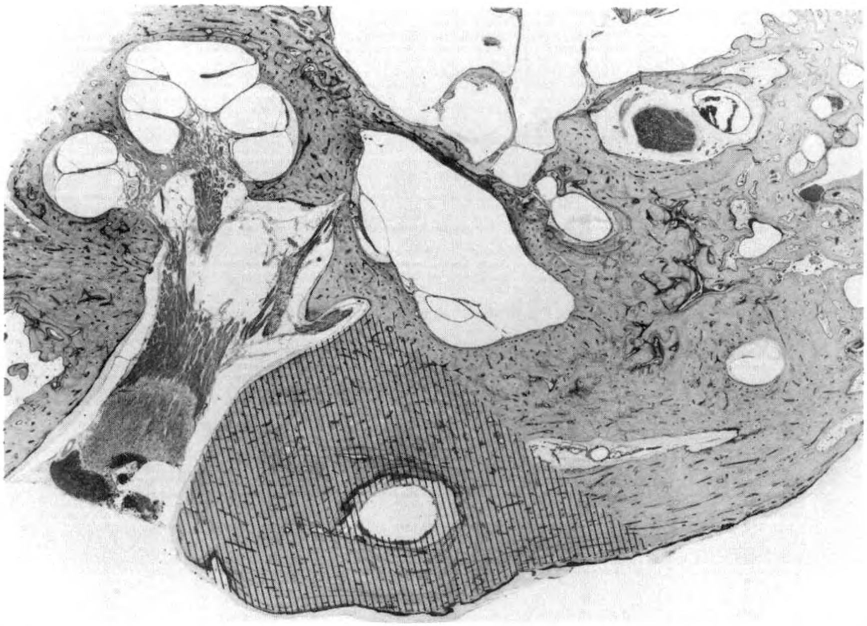

Fig 5. Horizontal section of right human temporal bone showing area of bone removal in suboccipital-retrosigmoid approach to internal auditory canal (original x8.3). Hatched area represents theoretic area of maximal bone removal. In 85% of specimens, apex of dome of jugular bulb fell within this wedge, bounded medially by posterior fossa dura, laterally by vestibular aqueduct and posterior semicircular canal, and anteriorly by internal auditory canal.

anatomic landmarks exist from which to measure. It is conceivable that estimates of this medial-lateral relationship could be made from vertical sections or coronal CT scans, but the same problem of consistent medial and/or lateral landmarks exists. However, by superimposition of slides containing the JB, crus commune, posterior semicircular canal, and posterior wall of the IAC, it was possible to get a visual estimate of the location of the dome of the JB relative to the boundaries of the wedge of bone that would be removed in the surgical exposure of the IAC (Fig 5). In 85% of high-JB specimens the dome fell within this wedge. In the other 15% it was positioned far enough laterally or posteriorly that it would not have been encountered surgically.

Three-dimensional reconstruction with rotation significantly aided study of the proximity of the JB to the IAC and provided greater appreciation of variations in the shape of the dome of the JB. Since histologic sections were obtained in the midmodiolar plane, they were tangential to the apex of the dome, making determination of the exact location difficult. It became clear from the computer reconstructions that some domes were broad and rather flat, while others were nearly conical, suggesting the diverticulum appearance described by Stern and Goldenberg[12] and Wadin et al.[15,16] These latter, conical domes were so narrow at their apex that we often had to scan back and forth through several serial sections to distinguish them from air cells.

DISCUSSION

The LVS and JB course sinuously from the posterosuperior to the anteroinferior aspect of the temporal bone. There is variability in the length of LVS encased within bone, in the curvature of the sigmoid portion of the LVS as it sweeps down and forward into the JB, and in the shape, size, and location of the JB itself (Fig 6). A "first genu" of the sigmoid sinus is formed at the distal limit of the LVS where the vessel swings anteriorly, and a "second genu" at the JB. However, the radius of curvature, diameter, and medial-lateral position at these sites and between them is highly variable. The course of this major vascular structure may change with maturation of the temporal bone itself. Our observation of high JB in only 2 of 32 (6%) specimens from children below 6 years of age compared to high JB in 47 of 75 (63%) of older children and adults would suggest that the tortuous upward deviation of the JB tends to form later in life. We were unable to more accurately correlate high JB development with age because of the fact that our temporal bone specimens tended to be from either very young or elderly patients, with few young and middle-aged adults. This sample bias reflects the demographics of mortality in the population donating temporal bones to our collection.

Based on review of temporal bone histopathologic specimens, Graham[9] proposed that the height and shape of the JB were related to pneumatization of the temporal bone. He speculated that a well-pneumatized temporal bone would tend to have a gently curving, posteriorly placed sigmoid sinus and a low JB, while poor pneumatization would be associated with an anteriorly placed, tightly curving sigmoid and a high JB. This hypothesis was supported by Kennedy et al[14] and by Wadin et al.[15,16] However, in a cadaver study correlating mastoid pneumatization as judged by plain radiographs or CT with JB shape and location as determined by dissection, Orr and Todd[17] re-

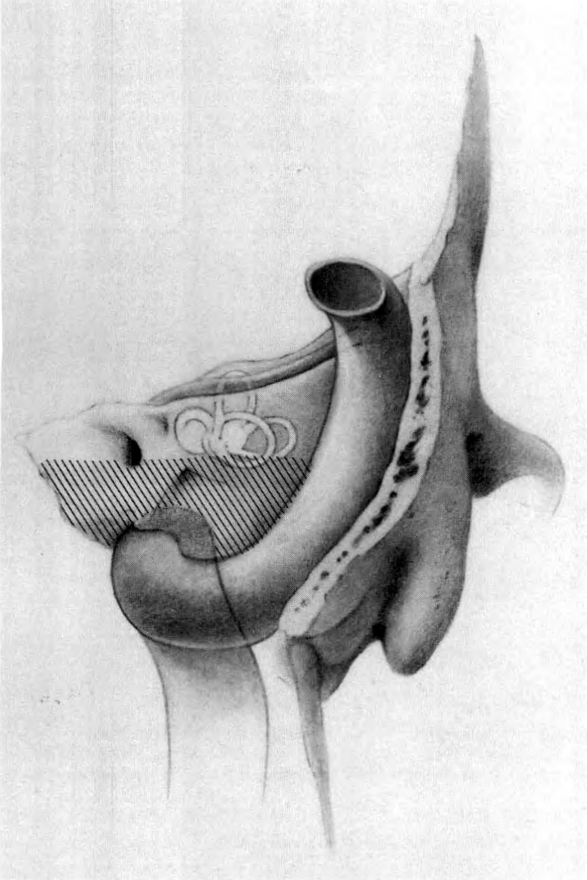

Fig 6. Artist's rendering of posterior aspect of right temporal bone. Jugular bulb may be found anywhere within hatched area.

futed this hypothesis. Specifically, they observed no correlation of JB configuration with degree of pneumatization in 25 cadaver specimens.

The definition of a high JB varies among studies. Graham[8,9] and others[7] have defined a high JB as extending to or above the inferior tympanic annulus, and report an incidence in the middle ear and hypotympanum of 5% to 10%, whereas Wadin et al[15,16] refer to the level of the round window niche. Wadin et al[15,16] required radiographic documentation of a high JB, whereas other authors[2,5,14,18] have relied on visual inspection of the middle ear, bulb, and tympanic annulus. Anson,[19] striving to quantify the anatomic relations, used posterior fossa landmarks for his large series of temporal bone measurements. Orr and Todd[17] note that most studies of JB position tend to be qualitative or of limited surgical applicability. They discovered that both the direct and vertical distances between the apex of the JB and the lateral extent of the IAC calculated by measurement from high-resolution CT scans are closely correlated with the actual vertical distance from the apex of the jugular dome to the plane of the horizontal semicircular canal as determined by dissection of cadaver specimens. This correlation held regardless of whether the apex of the JB dome was inferior, anterior, lateral, posterior, or medial to the lateral apex of the IAC.[17] Unfortunately, from the posterior fossa approach there is no landmark for the location of the plane of the horizontal semicircular canal.

In our present study of human temporal bone specimens we chose to use a clinically applicable definition of high JB not previously described: placement of the dome of the JB within 2.0 mm of the inferiormost limit of the internal auditory meatus. With this definition we found a high JB in nearly two thirds of specimens from older children and adults — a far higher incidence than in any other report using anatomic landmarks in the middle ear. This corroborates our clinical impression that the JB is prone to exposure in the majority of procedures requiring osteotomy of the IAC by the suboccipital approach.

We have not attempted to catalog or describe all the variations in anatomy of the sigmoid sinus–JB complex. Our objective in this study was to evaluate the proximity of the JB to the IAC, which is relevant to the posterior fossa surgical approach. From a surgical perspective, a JB within 2 mm of the IAC may well be exposed and possibly injured in efforts to skeletonize the posterior wall of the IAC. From a practical perspective, 2 mm correlates well with the distance between slices on a high-resolution CT scan, and the midmodiolar plane of section of our temporal bone specimens corresponds almost precisely to the plane of an axial CT scan used in clinical radiology. Therefore, a JB within 2 mm of the lower limit of the IAC can be diagnosed by the finding of the IAC and JB on the same or adjacent cuts of a CT scan. We now routinely survey preoperative CT scans for these landmarks. If the JB is visualized in the CT panel adjacent to the lowest one showing the IAC, we expect that the bulb may be encountered surgically. If the IAC and JB appear in the same plane, we are virtually certain to expose it (Fig 7).

Magnetic resonance imaging with gadolinium enhancement has become the standard diagnostic study for delineation of acoustic neuroma or other cerebellopontine mass lesions. However, magnetic resonance imaging provides inadequate detail of bone and the sigmoid sinus and JB because of the "flow void." Surgical exposure provided by the suboccipital approach reveals the JB, like the posterior semicircular canal and/or crus commune, tangentially rather than en face (Fig 4B). If the sigmoid segment of the LVS is encased in bone, there is no hint of the precise location of the bulb until it is "blue-lined" or exposed. Information from a preoperative CT scan aids the surgeon in anticipating the location of the JB and

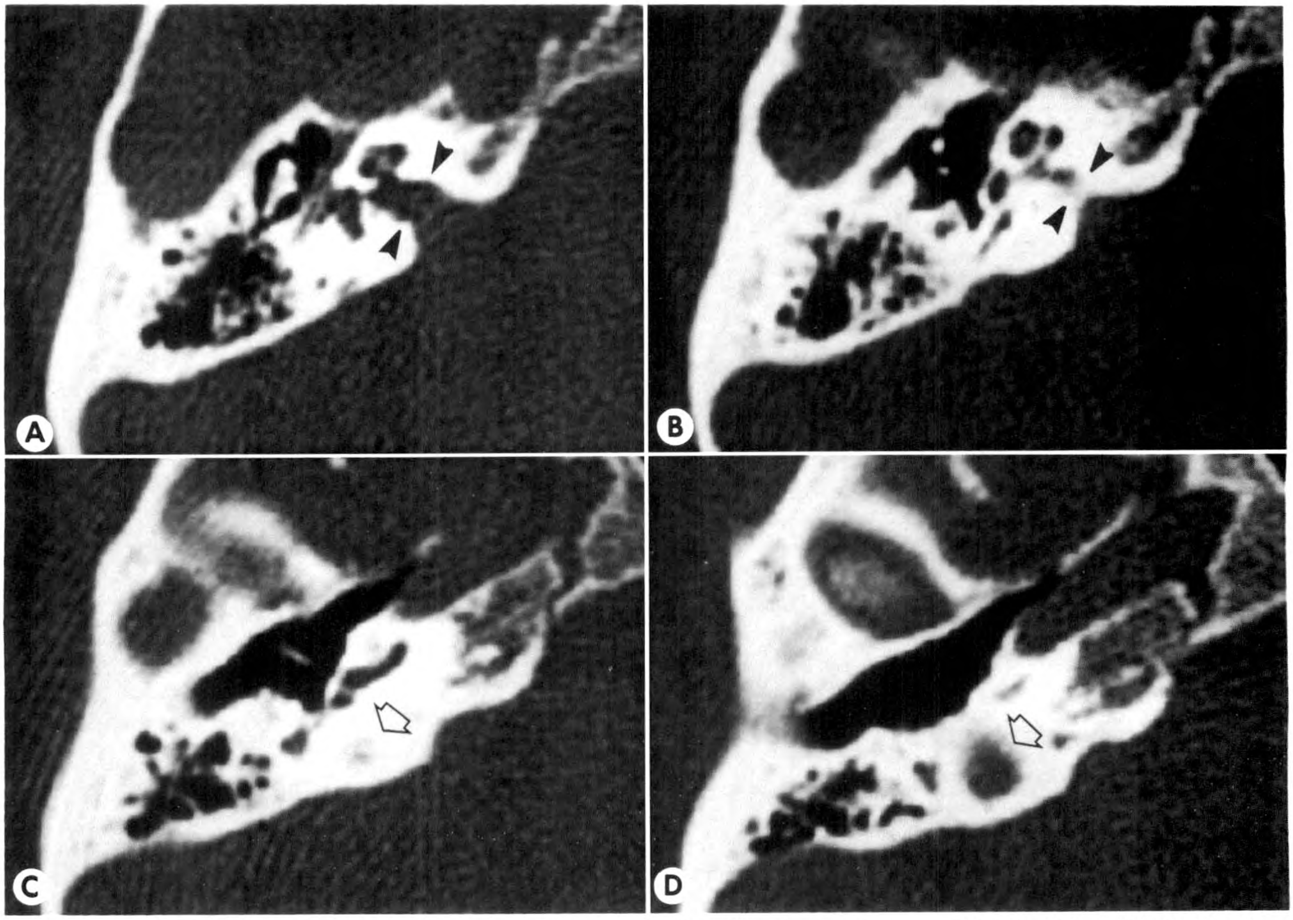

Fig 7. Axial computed tomograms of temporal bone. Four consecutive slices demonstrate apex of jugular bulb and lower lip of internal auditory meatus in adjacent "cuts." In such cases, bulb is likely to be exposed surgically during bone removal. Arrowheads — internal auditory canal, open arrows — jugular bulb.

thereby avoiding injury to it. In occasional cases, the CT findings will even lead to alteration in the surgical approach in order to skeletonize and compress or displace the entire sigmoid sinus and JB in those cases in which they obstruct adequate exposure of the IAC via the posterior fossa.

ACKNOWLEDGMENT — The authors thank medical illustrator Robert J. Galla.

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20TH INTERNATIONAL CONFERENCE ON COCHLEAR IMPLANTS

The 20th International Conference on Cochlear Implants will be held June 23-25, 1993, at the Ritz-Carlton Hotel, San Francisco, California. For further information, contact University of California, Extended Programs in Medical Education, Room LS-105, San Francisco, CA 94143-0742; (415) 476-4251.

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1993 CONFERENCE ON IMPLANTABLE AUDITORY PROSTHESES

The 1993 Conference on Implantable Auditory Prostheses will be held July 11-15, 1993, on the campus of Bryant College in Smithfield, Rhode Island. This is the sixth in a series of biennial research conferences originating with the 1983 Gordon Conference. The meeting will provide a forum for close interaction among scientists in all disciplines relating to the field of cochlear implants. For further information, contact Chris van den Honert, Division of Otolaryngology–Head & Neck Surgery, Box 3550, Duke University Medical Center, Durham, NC 27710; Telephone: (919) 684-6099, E-Mail: chx@dukehrl.mc.duke.edu.

Ann Otol Rhinol Laryngol 102:1993

INTRACRANIAL LONGITUDINAL SPLITTING OF FACIAL NERVE: A NEW APPROACH FOR HEMIFACIAL SPASM

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A new surgical technique for the treatment of hemifacial spasm consisting of longitudinal splitting of the facial nerve in the cerebellopontine angle is described. Thirty-three cases have been treated with good results. Follow-up of 20 cases for 1 year or more showed no recurrence or facial paresis. One case required a second operation. The novelty of this approach lies in its effectiveness in relieving the symptoms without running the risk of overmanipulating the aberrant artery.

KEY WORDS — hemifacial spasm, microvascular decompression.

Hemifacial spasm manifesting as synkinesis may become an intolerable disease. Lesions in the brain affecting the facial nerve nucleus, narrowing of the nerve pathway through the temporal bone, and compression of the nerve by a space-occupying lesion or vascular loop have all been suggested in various cases as the cause. Despite extensive efforts, the cause in come cases remains unknown. As medical treatment often is of no avail, surgical procedures are resorted to: among others, selective neurotomy, partial sectioning of the facial nerve, and the use of a wire loop for ligature of the facial nerve peripherally. The last procedure, reported by the author in 1982,[1] usually resulted in transitory facial paralysis, and there was a recurrence rate of about 30%. Jannetta[2] recommended microvascular decompression as early as 1967, but the procedure was more widely accepted only in recent years. My own experience with this technique has been fair, but about 10% of my cases ended in failure. Subsequently, I began to use a new approach consisting of splitting of the facial nerve root. Thirty-three cases have been treated this way satisfactorily.

TECHNIQUE

The patient is put in the contralateral decubitus position and is preferably under general anesthesia. (Local anesthesia may induce dizziness and vomiting.) A postsigmoid craniotomy is performed through an elliptical incision behind the mastoid. The cerebellum is separated away from cranial nerves IX and X. After exposure of cranial nerves VII and VIII, the crossing vessel can usually be seen. Enclosing arachnoidal bands and adhesive perineural tissue are stripped away from the nerve trunk. The facial nerve is transfixed with a thin, sharp knife, and one or more longitudinal cuts are made (see Figure, A). The nerve is thus longitudinally split into two or more bundles within a segment about 0.5 cm long. The crossing vessel is left alone, without mobilization or insertion of tissue in between. Microsurgical techniques should be observed throughout the operation.

CLINICAL DATA AND COMMENT

In this series of cases there were 15 men and 18 women ranging from 18 to 66 years of age. The duration of symptoms was 2 to 21 years. All patients had sought medical treatment without result prior to surgery. A vascular loop was readily found in 24 cases compressing the seventh nerve root at the exit zone. The vascular loop was situated anteriorly in 9 cases and posteriorly in 11 cases. In 4 cases the artery traversed between cranial nerves VII and VIII anterocaudally or posterocephalad. The results have been excellent in 26 cases, with no residual spasm or weakness. Six patients had very mild spasm remaining, and it disappeared completely after 3 to 13 days. There was 1 case that required a second operation. Complete relief was achieved by making more longitudinal cuts. Follow-up of 20 patients for 1 year or more showed no recurrence in all except 1, who had a very mild lower eyelid spasm.

Microvascular decompression was an effective treatment for hemifacial spasm in most cases, but there was an apparent discrepancy occasionally between clinical improvement and the degree of decompression. Jannetta pointed out,

> If the facial nerve is not touched during the operation, the patient awakens from anesthesia with some hemifacial spasm which then gradually disappears. If the nerve is gently struck or manipulated during the procedure the patient usually awakens from anesthesia permanently free of spasm.[3]

A similar idea was expressed by Sweet.[4] Appar-

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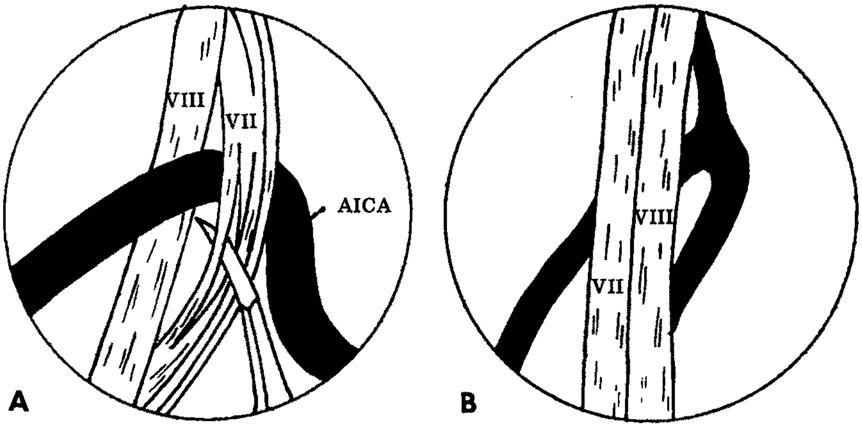

A) Facial nerve being split into bundles. AICA — anterior inferior cerebellar artery. B) Internal auditory artery arising from top of arterial loop and entering porus acusticus.

ently, gentle manipulation of the nerve was useful in relief. I was thus led to develop a technique something like a gentle manipulation of the involved nerve. There were still some cases that failed to achieve complete relief. Then I started to make longitudinal cuts of the nerve, a procedure described as combing (see Figure, A). It was found that this procedure alone was sufficient for the relief of spasm without running the risk of microvascular dissection. The procedure serves at least as an alternative when the compressing vascular loop is difficult to locate or the dissection of the arterial loop is too risky. In one of our cases the internal auditory artery was found to originate from the top of the loop (see Figure, B). Excessive manipulation might cause ataxia or ipsilateral hearing loss.

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4TH INTERNATIONAL ACADEMIC CONFERENCE
IMMUNOBIOLOGY IN OTOLOGY, RHINOLOGY AND LARYNGOLOGY

The 4th international academic conference Immunobiology in Otology, Rhinology and Laryngology will be held in Oita, Japan, April 4-7, 1994. For further information contact Goro Mogi, MD, Dept of Otolaryngology, Oita Medical University, Hasama-machi, Oita 879-55, Japan; telephone 81-975-49-4411 ext 2912 or 2913; fax 81-975-49-0762.

Ann Otol Rhinol Laryngol 102:1993

COMPLIANCE OF THE PATULOUS EUSTACHIAN TUBE

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The compliance and ventilatory functions of the eustachian tube (ET) of 17 ears with patulous ETs were examined and compared to those of 16 ears with traumatic perforation of the eardrum (controls) by means of the tubal compliance test, the inflation-deflation test, and the forced response test. The tubal compliance was significantly lower in the patulous ETs than in the controls. An excessive patency of the ET was confirmed among the patients with patulous ETs by a low opening pressure during the inflation test and a low passive resistance on the forced response test. Active ventilatory dysfunction was also found to be significantly more common in the patulous ETs by the deflation test and the measure of dilation efficiency. These results indicated that the ET appeared to be rather rigid and less moveable when patulous than when normal.

KEY WORDS — eustachian tube function, patulous eustachian tube, tubal compliance.

INTRODUCTION

Patulous eustachian tube (ET) is an intractable ET condition with uncomfortable symptoms such as autophony and/or a stuffy sensation in the ear. It is often triggered by rapid weight loss, pregnancy, contraceptive pills, and some neuromuscular diseases.[1] Furthermore, some patulous ETs have been reported in conjunction with middle ear complications such as otitis media with effusion (OME), atelectatic eardrum, and middle ear cholesteatoma.[2] In spite of its intractability and association with the serious complications mentioned above, the pathophysiology of the ET in this condition has not been fully investigated.

We examined the compliance[3] and the ventilatory functions of the ETs in patients with patulous ETs to obtain insight into the pathophysiology of this condition and possibly to refine its medical and surgical therapy.

MATERIALS AND METHODS

Participants in this study consisted of 14 individuals (17 ears) 13 to 76 years of age (mean 44.5 ± 25.2) with patulous ET syndrome as diagnosed by changes in the position of the eardrum due to respiration, and 19 controls (22 ears) 9 to 41 years of age (mean 23.7 ± 12.5) with traumatic perforation of the eardrum and without a history of any other otologic disease.

After informed consents were obtained from the patients, myringotomy was performed. The ET functions were then examined by the inflation-deflation test and the forced response test developed by Cantekin et al.[4,5] Passive tubal functions were characterized by the opening pressure and the passive tubal resistance at three airflow rates (10, 20, and 40 mL/min). Active tubal functions were defined by the negative middle ear pressure (−150 to −200 mm H_2O) equalizing function and by the dilation efficiency of Cantekin et al,[4,5] defined as the ratio of the passive resistance at an airflow rate of 20 mL/min to the active resistance value during swallowing. The compliance of the ET, evaluated as the tubal compliance index,[3] was obtained as the ratio of the two passive resistance values at airflow rates of 10 mL/min and 40 mL/min. Resistance is affected by the length and caliber of the tube. The change in passive resistance of the ET with the change in airflow rate is considered to be due to a change in caliber of the ET, because the length of the ET cannot change.[3] The degree of the change in the passive resistance therefore indicates distensibility of the ET — in other words, compliance of the ET.[3]

RESULTS

Figure 1 shows the passive resistance of the ET in both groups at the airflow rates of 10, 20, and 40 mL/min. With the increase of the airflow rate from 10 mL/min to 40 mL/min, a significant decrease in the passive resistance was seen in the control group (Wilcoxon's ranking test, $t = 621.5$, $p < .01$), whereas the decrease was not statistically significant in the patulous ET group. Most of the patulous ET ears also showed lower tubal resistance than the controls at all the airflow rates (Fig 1). The difference of passive

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Presented at the Fifth International Symposium on Recent Advances in Otitis Media, Fort Lauderdale, Florida, May 20-24, 1991.

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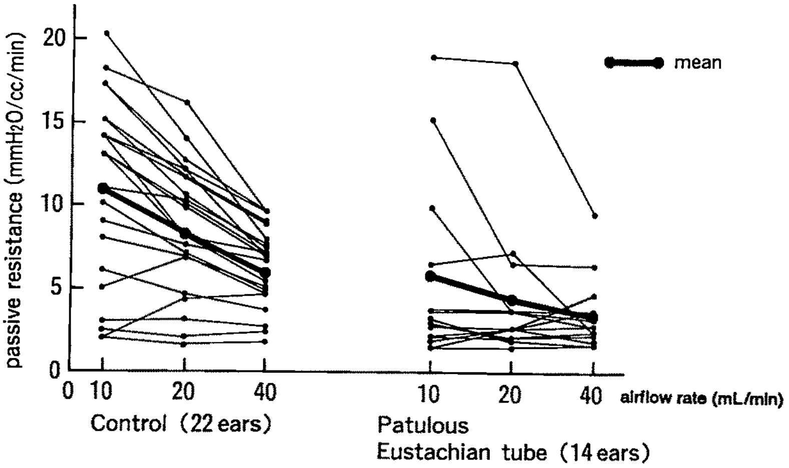

Fig 1. Passive tubal resistance of eustachian tube at three airflow rates.

resistance was statistically significant at all the airflow rates (10 mL/min, $t = 189.0$, $p < .05$; for 20 mL/min, $t = 169.0$, $p < .01$; for 40 mL/min, $t = 173.5$, $p < .01$).

As shown in Fig 2, the tubal compliance index was found to be significantly lower in the patulous ET group than in the control group (Wilcoxon's ranking test, $t = 207.0$, $p < .05$).

As evidenced by the inflation test (Fig 3), the opening pressure was significantly lower in the patulous ET group than in the control group (Wilcoxon's ranking test, $t = 136.5$, $p < .01$).

In the deflation test, negative pressure was not maintained in the middle ear in 9 of 17 ears (52.9%) with patulous ETs. Among the remaining 8 ears, only 4 (50%) achieved negative pressure equalization during swallowing. All 19 ears of the control group could equilibrate the negative middle ear pressure (χ^2 test, $p < .01$). In the forced response test, as shown in Fig 4, the dilation efficiency was significantly lower in the patulous ETs than in the control group (Wilcoxon's ranking test, $t = 132.5$, $p < .01$). In 1 of the 14 ears with patulous ETs, the active dilation index was less than 1, indicating that the ET constricted during swallowing.

DISCUSSION

The present results of the compliance study of the ET indicated that most of the patulous ETs were

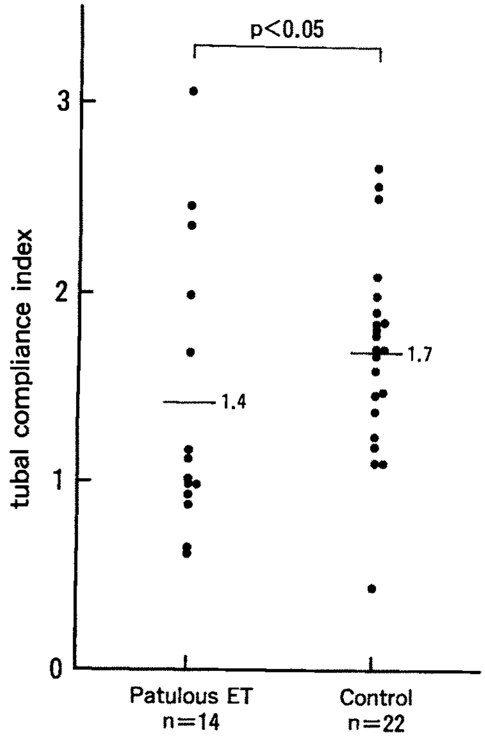

Fig 2. Tubal compliance indices of two groups.

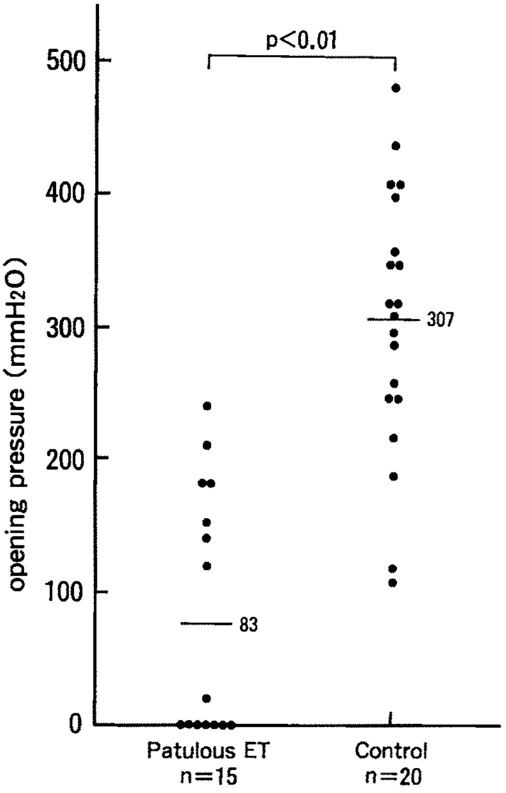

Fig 3. Passive opening pressure of eustachian tube.

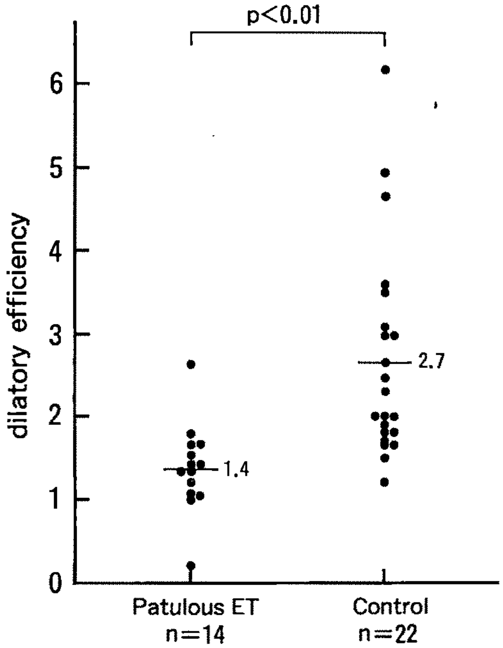

Fig 4. Dilation efficiency of two groups at airflow rate of 20 mL/min.

likely to be rigid rather than hypercompliant. The patulous condition of the ET can be caused by rapid weight loss or some neuromusular diseases,[1] and is often seen in aged populations as well. Rapid weight loss may affect the volume of Ostmann's fatty tissue, located lateral to the tubal lumen. Atrophy of the peritubal muscles (the tensor and levator veli palatini muscles) has been reported in older individuals,[6] and in neuromuscular diseases as well. The low compliance of the ET in patients with patulous ETs revealed in this study, therefore, may be attributed partly to a loss of elasticity of the ET due to the decrease of the volume of these surrounding soft tissues.

Probably because of the difficulty in measuring the active functions of the ET due to its excessive patency, only a few reports have systematically investigated the tubal function in patulous ET syndrome.[7,8] Virtanen[7] examined 25 cases of patulous ET by sonotubometry, and noted a prolonged tubal opening after swallowing, indicating the closing failure of the ET in most of these cases. The abnormally low opening pressures and low passive tubal resistances in the patulous ET group in our study are consistent with the excessive patency postulated by Virtanen.

Half of the patulous ET ears failed to equilibrate their negative middle ear pressure during swallowing, indicating poor ability to actively dilate the ET. This lack of dilation efficiency also revealed that dynamic tubal function was markedly impaired in the patulous ET group. Virtanen[7] found one case with poor negative pressure equilibration out of three patulous ET cases examined, and attributed this observation to poor muscular dilation. Active ventilatory dysfunction is thought to be one of the pathologic conditions among patulous ET cases.

On the other hand, in only one (7%) of the patulous ET cases examined was the active dilation index less than 1, implying a constriction of the ET during swallowing. Such paradoxical movement of the ET was reported in 22.7% of pediatric OME cases and in 39.1% of adult OME cases by Takahashi et al,[9] and in as many as 82.5% of pediatric OME cases by Lildholdt et al.[10] Constriction of the ET during swallowing, therefore, seemed to be less common in the patulous ET.

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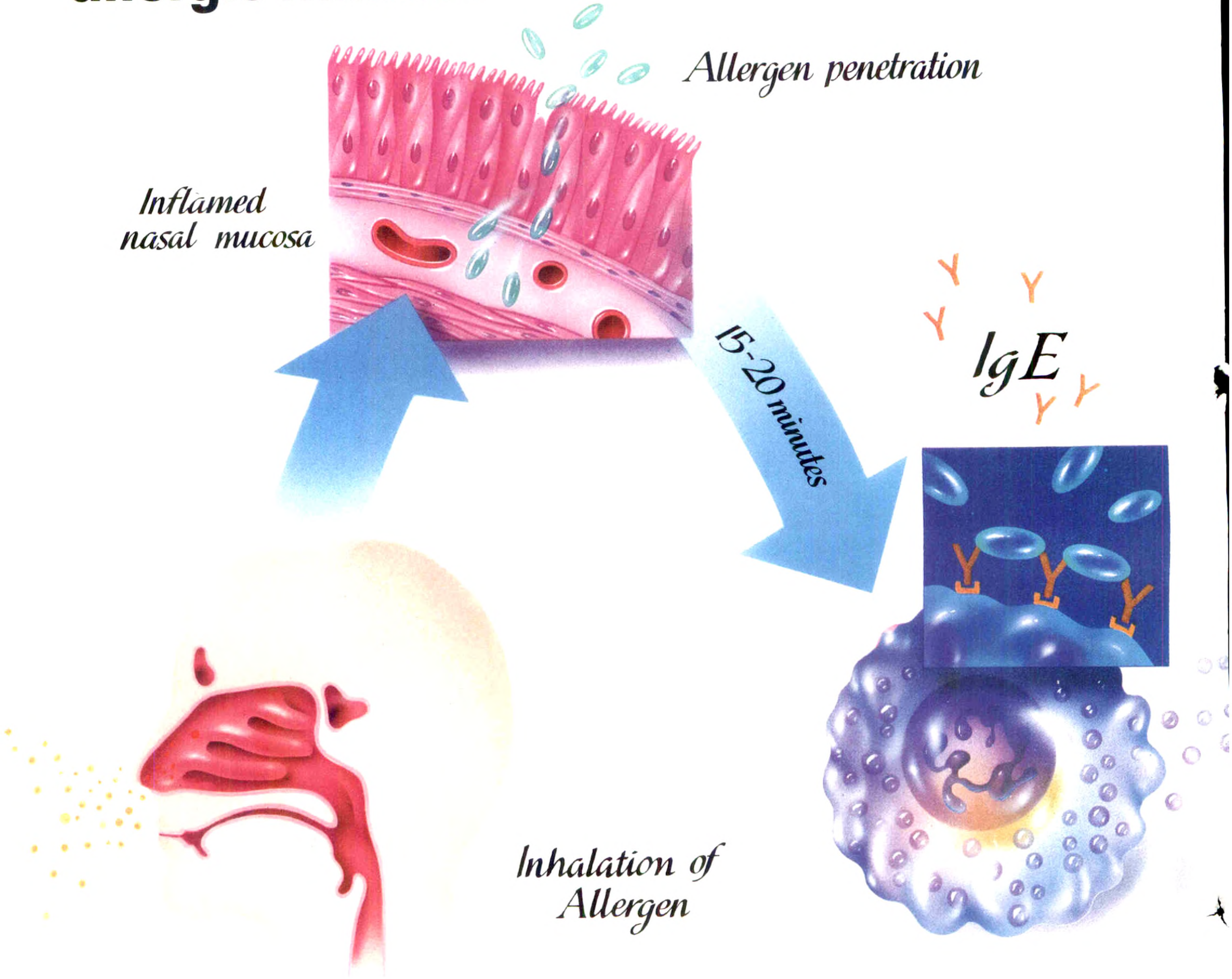

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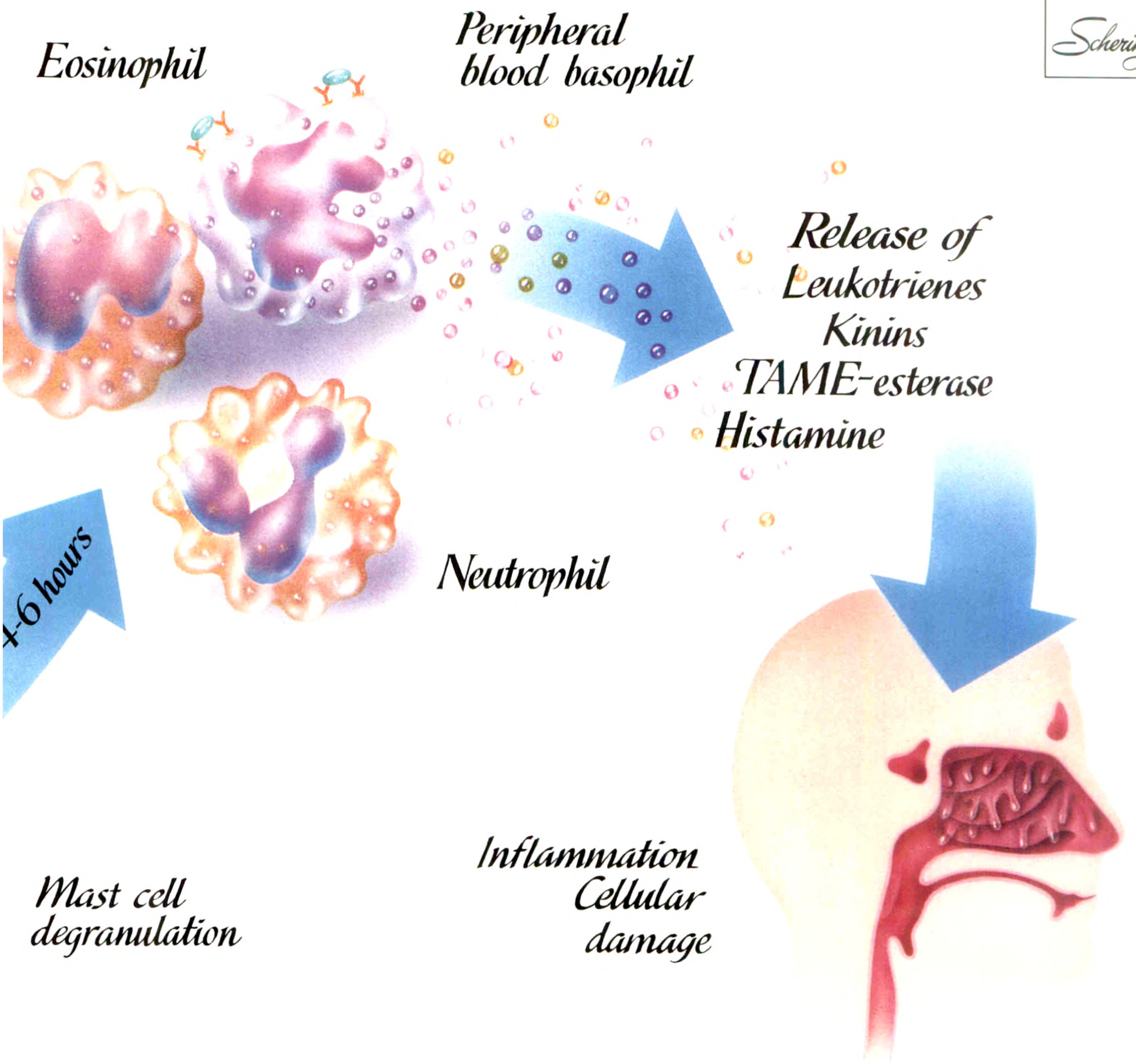

Water-based b.i.d.

VANCENASE® AQ

brand of beclomethasone dipropionate, monohydrate **Nasal Spray, 0.042%***

*Calculated on the dried basis.

VANCENASE® POCKETHALER®

(beclomethasone dipropionate, USP) **Nasal Inhaler**

VANCENASE POCKETHALER Nasal Inhaler is indicated for the relief of the symptoms of seasonal or perennial rhinitis in those cases poorly responsive to conventional treatment.
Patients in this study (n=161) used beclomethasone dipropionate aqueous spray.
No significant difference from placebo in incidence of side effects in a study of 101 children 6 to 12 years old. The most frequent side effects with beclomethasone dipropionate AQ in this study[4] were transient burning or stinging in the nose, and headache.

Please see next page for brief summary of prescribing information.

References: 1. Naclerio RM. Allergic rhinitis. *N Engl J Med.* 1991;325(12):860-869. **2.** Juniper EF, Kline PA, Hargreave FE, Dolovich J. Comparison of beclomethasone dipropionate aqueous nasal spray, astemizole, and the combination in the prophylactic treatment of ragweed pollen-induced rhinoconjunctivitis. *J Allergy Clin Immunol.* 1989;83:627-633. **3.** Bronsky EA, Tarpay M, Tinkelman DA, Bush JK. A comparison of two dosing regimens of beclomethasone dipropionate aqueous nasal spray and flunisolide nasal spray in the treatment of acute seasonal rhinitis. *Immunol Allergy Pract.* 1987;9(5):11-16. **4.** Kobayashi RH, Tinkelman DG, Reese ME, Sykes RS, Pakes GE. Beclomethasone dipropionate aqueous nasal spray for seasonal allergic rhinitis in children. *Ann Allergy.* 1989;62:205-208.

brand of beclomethasone dipropionate, monohydrate

Nasal Spray 0.042%*
FOR INTRANASAL USE ONLY

***calculated on the dried basis**

BRIEF SUMMARY
(For full Prescribing Information see package insert)

INDICATIONS AND USAGE VANCENASE AQ Nasal Spray is indicated for the relief of the symptoms of seasonal or perennial allergic and non-allergic (vasomotor) rhinitis. Results from two clinical trials have shown that significant symptomatic relief was obtained within three days. However, symptomatic relief may not occur in some patients for as long as two weeks. VANCENASE AQ Nasal Spray should not be continued beyond three weeks in the absence of significant symptomatic improvement. VANCENASE AQ Nasal Spray should not be used in the presence of untreated localized infection involving the nasal mucosa.

VANCENASE AQ Nasal Spray is also indicated for the prevention of recurrence of nasal polyps following surgical removal.

Clinical studies have shown that treatment of the symptoms associated with nasal polyps may have to be continued for several weeks or more before a therapeutic result can be fully assessed. Recurrence of symptoms due to polyps can occur after stopping treatment, depending on the severity of the disease.

CONTRAINDICATIONS Hypersensitivity to any of the ingredients of this preparation contraindicates its use.

WARNINGS The replacement of a systemic corticosteroid with VANCENASE AQ Nasal Spray can be accompanied by signs of adrenal insufficiency.

When transferred to VANCENASE AQ Nasal Spray, careful attention must be given to patients previously treated for prolonged periods with systemic corticosteroids. This is particularly important in those patients who have associated asthma or other clinical conditions, where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate day prednisone systemic treatment and orally inhaled beclomethasone increased the likelihood of HPA suppression compared to a therapeutic dose of either one alone. Therefore, VANCENASE AQ Nasal Spray treatment should be used with caution in patients already on alternate day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, VANCENASE AQ Nasal Spray should be discontinued slowly, consistent with accepted procedures for discontinuing oral steroid therapy.

PRECAUTIONS **General:** During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, e.g., joint and/or muscular pain, lassitude, and depression. Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone.

Extremely rare instances of wheezing, nasal septum perforation and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids. Although these have not been observed in clinical trials with VANCENASE AQ Nasal Spray, vigilance should be maintained.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuance of treatment with VANCENASE AQ Nasal Spray.

If persistent nasopharyngeal irritation occurs, it may be an indication for stopping VANCENASE AQ Nasal Spray.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of VANCENASE AQ Nasal Spray may suppress HPA function.

VANCENASE AQ Nasal Spray should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract, or in untreated fungal, bacterial, systemic viral infections, or ocular herpes simplex.

For VANCENASE AQ Nasal Spray to be effective in the treatment of nasal polyps, the spray must be able to enter the nose. Therefore, treatment of nasal polyps with VANCENASE AQ Nasal Spray should be considered adjunctive therapy to surgical removal and/or the use of other medications which will permit effective penetration of VANCENASE AQ Nasal Spray into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using VANCENASE AQ Nasal Spray over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septal ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses should be avoided.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrous cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrous cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy Category C: Like other corticosteroids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately ten times the human dose. In these studies beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at ten times the human dose or orally at 1000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when VANCENASE AQ Nasal Spray is administered to nursing women.

Pediatric Use: Safety and effectiveness in children below the age of 6 years have not been established.

ADVERSE REACTIONS In general, side effects in clinical studies have been primarily associated with irritation of the nasal mucous membranes. Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone dipropionate.

Adverse reactions reported in controlled clinical trials and open studies in patients treated with VANCENASE AQ Nasal Spray are described below.

Mild, transient nasopharyngeal irritation following the use of beclomethasone aqueous nasal spray has been reported in up to 24% of patients treated, including occasional sneezing attacks (about 4%) occurring immediately following use of the inhaler. In patients experiencing these symptoms, none had to discontinue treatment. The incidence of irritation and sneezing was approximately the same in the group of patients who received placebo in these studies, implying that these complaints may be related to vehicle components of the formulation.

Fewer than 5 per 100 patients reported headache, nausea or lightheadedness following the use of VANCENASE AQ (beclomethasone dipropionate, monohydrate) Nasal Spray. Fewer than 3 per 100 patients reported nasal stuffiness, nosebleeds, rhinorrhea and tearing eyes.

Extremely rare instances of wheezing, nasal septum perforation and increased intraocular pressure have been reported following the intranasal administration of aerosolized corticosteroids (see **PRECAUTIONS**).

OVERDOSAGE When used at excessive doses, systemic corticosteroid effects such as hypercorticism and adrenal suppression may appear. If such changes occur, VANCENASE AQ Nasal Spray should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy. The oral LD_{50} of beclomethasone dipropionate is greater than 1 g/kg in rodents. One bottle of VANCENASE AQ Nasal Spray contains beclomethasone dipropionate, monohydrate equivalent to 10.5 mg of beclomethasone dipropionate; therefore, acute overdosage is unlikely.

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Revised 8/88

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16102202-JBS

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brand of beclomethasone dipropionate, USP

POCKETHALER® Nasal Inhaler
For Nasal Inhalation Only

BRIEF SUMMARY
(For full Prescribing Information see package insert)

INDICATIONS AND USAGE VANCENASE POCKETHALER Nasal Inhaler is indicated for the relief of the symptoms of seasonal or perennial rhinitis in those cases poorly responsive to conventional treatment.

VANCENASE POCKETHALER Nasal Inhaler is also indicated for the prevention of recurrence of nasal polyps following surgical removal.

Clinical studies in seasonal and perennial rhinitis have shown that improvement is usually apparent within a few days. However, symptomatic relief may not occur in some patients for as long as 2 weeks. Although systemic effects are minimal at recommended doses, VANCENASE treatment should not be continued beyond 3 weeks in the absence of significant symptomatic improvement. VANCENASE treatment should not be used in the presence of untreated, localized infection involving the nasal mucosa.

Clinical studies have shown that treatment of the symptoms associated with nasal polyps may have to be continued for several weeks or more before a therapeutic result can be fully assessed. Recurrence of symptoms due to polyps can occur after stopping treatment, depending on the severity of the disease.

CONTRAINDICATIONS Hypersensitivity to any of the ingredients of this preparation contraindicates its use.

WARNINGS The replacement of a systemic corticosteroid with VANCENASE POCKETHALER Nasal Inhaler can be accompanied by signs of adrenal insufficiency.

Careful attention must be given when patients, previously treated for prolonged periods with systemic corticosteroids, are transferred to VANCENASE POCKETHALER Nasal Inhaler. This is particularly important in those patients who have associated asthma or other clinical conditions, where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate day prednisone systemic treatment and orally inhaled beclomethasone increased the likelihood of HPA suppression compared to a therapeutic dose of either one alone. Therefore, VANCENASE treatment should be used with caution in patients already on alternate day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, VANCENASE POCKETHALER Nasal Inhaler should be discontinued slowly, consistent with accepted procedures for discontinuing oral steroid therapy.

Children who are on immunosuppressant drugs are more susceptible to infections than healthy children. Chickenpox and measles, for example, can have a more serious or even fatal course in children on immunosuppressant corticosteroids. In such children, or in adults who have not had these diseases, particular care should be taken to avoid exposure. If exposed, therapy with varicella zoster immune globulin (VZIG) or pooled intravenous immunoglobulin (IVIG), as appropriate, may be indicated. If chickenpox develops, treatment with antiviral agents may be considered.

PRECAUTIONS General: During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, eg, joint and/or muscular pain, lassitude, and depression.

Extremely rare instances of nasal septum perforation and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuance of treatment with VANCENASE POCKETHALER Nasal Inhaler.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of VANCENASE POCKETHALER Nasal Inhaler may suppress HPA function.

VANCENASE treatment should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract, or in untreated fungal, bacterial, systemic viral infections, or ocular herpes simplex.

For VANCENASE POCKETHALER Nasal Inhaler to be effective in the treatment of nasal polyps, the aerosol must be able to enter the nose. Therefore, treatment of nasal polyps with VANCENASE Nasal Inhaler should be considered adjunctive therapy to surgical removal and/or the use of other medications which will permit effective penetration of the VANCENASE product into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using VANCENASE treatment over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septum ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses should be avoided.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrus cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrus cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy Category C: Like other corticoids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately ten times the human dose. In these studies, beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at ten times the human dose or orally at 1000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when VANCENASE POCKETHALER Nasal Inhaler is administered to nursing women.

Pediatric Use: Safety and effectiveness in children below the age of 6 years have not been established.

ADVERSE REACTIONS In general, side effects in clinical studies have been primarily associated with the nasal mucous membranes. Adverse reactions reported in controlled clinical trials and in long-term open studies in patients treated with VANCENASE Nasal Inhaler are described below.

Sensations of irritation and burning in the nose (11 per 100 patients) following the use of VANCENASE Nasal Inhaler have been reported. Also, occasional sneezing attacks (10 per 100 patients) have occurred immediately following the use of the intranasal inhaler. This symptom may be more common in children.

Rhinorrhea may occur occasionally (1 per 100 patients).

Localized infections of the nose and pharynx with *Candida albicans* have occurred rarely. (See **PRECAUTIONS.**)

Transient episodes of epistaxis or bloody discharge from the nose have been reported in 2 per 100 patients.

Ulceration of the nasal mucosa has been reported rarely.

Extremely rare instances of nasal septum perforation have been reported following the intranasal application of aerosolized corticosteroids. Rare cases of immediate and delayed hypersensitivity reactions, including urticaria, angioedema, rash, and bronchospasm have been reported following the oral and intranasal inhalation of beclomethasone.

Increased intraocular pressure has been reported rarely. (See **PRECAUTIONS.**)

Systemic corticosteroid side effects were not reported during controlled clinical trials. If recommended doses are exceeded, however, or if individuals are particularly sensitive, symptoms of hypercorticism, ie, Cushing's syndrome could occur.

OVERDOSAGE When used at excessive doses, systemic corticosteroid effects such as hypercorticism and adrenal suppression may appear. If such changes occur, VANCENASE POCKETHALER Nasal Inhaler should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy.

The oral LD_{50} of beclomethasone dipropionate is greater than 1 g/kg in rodents. One canister of VANCENASE POCKETHALER Nasal Inhaler contains 8.4 mg of beclomethasone dipropionate; therefore acute overdosage is unlikely.

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IMMUNE RESPONSE TO *PREVOTELLA INTERMEDIA* IN PATIENTS WITH RECURRENT NONSTREPTOCOCCAL TONSILLITIS

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The role of three oral flora organisms (*Prevotella intermedia, Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*) was investigated in 31 children with recurrent nonstreptococcal tonsillitis. Antibody titers to the three organisms were measured by enzyme-linked immunosorbent assay in the 31 patients, as well as in 32 control patients who had not suffered from recurrent tonsillitis. None of the individuals in either group suffered from periodontal or dental illness. Significantly higher antibody levels to *P intermedia* were found in the study group as compared to controls (median 91.0 versus 72.5; $p = .02$). In contrast, the antibody titers to the other two organisms were generally low (less than 0.30), and no difference was found among the two study groups. The elevated antibody levels to *P intermedia*, a known oral pathogen that is also isolated from most recurrently inflamed tonsils, suggest a pathogenic role for this organism in recurrent tonsillitis.

KEY WORDS — *Actinobacillus actinomycetemcomitans*, bacteriology, nonstreptococcal tonsillitis, *Porphyromonas gingivalis, Prevotella intermedia.*

INTRODUCTION

Recurrent tonsillitis is a common infectious problem in pediatric and adolescent patients. Although group A β-hemolytic streptococci (GABHS) can be isolated from 25% to 40% of patients,[1] other bacteria, usually called "normal oral flora," are isolated from the rest of the patients.

Anaerobic bacteria predominate in the normal oral flora, outnumbering aerobic flora in a ratio of 10 to 1, and are known to cause an acute tonsillar inflammation in the form of Vincent's angina.[2] The potential role of anaerobic bacteria in the recurrent inflammatory tonsillar process was investigated in patients with recurrent non-GABHS tonsillitis (RNST). The presence of antibodies of the immunoglobulin G class to three organisms commonly found in the oral flora (*Prevotella intermedia, Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*) was investigated by enzyme-linked immunosorbent assay (ELISA).

PATIENTS AND METHODS

Patients. Thirty-one patients (18 female and 13 male) who were seen consecutively in an outpatient otolaryngology clinic by one of us (P.A.F.) between September 1982 and June 1987 participated in the study group (group A). The mean age of patients was 19 years 6 months (range 12 to 51 years). The patients had been referred by primary care physicians to the clinic for elective tonsillectomy because they had suffered from RNST.

At the time of their initial presentation to the clinic, none of the patients was symptomatic. Criteria for inclusion in the study were as follows: 1) presence of RNST within the past 2 years, defined as the occurrence of at least three episodes annually of documented acute, moderate to severe pharyngeal and/or tonsillar inflammation; 2) failure to detect GABHS in pharyngotonsillar cultures from any of these episodes; 3) each episode characterized by two or more specific predetermined signs and symptoms (oral temperature above 38°C, enlarged and/or tender cervical lymph glands, and tonsillar or pharyngeal inflammation with or without an exudate). Excluded were patients who had a history of periodontal disease. Prior to surgery a serum sample was obtained from each individual. The serum samples were kept frozen at −70°C.

Serum samples were also obtained from 32 normal, arbitrarily selected individuals who served as controls (group B). These patients were admitted to the same hospital for a variety of elective surgical procedures, none of which involved inflammation or infection. The mean age (21 years 4 months) of these

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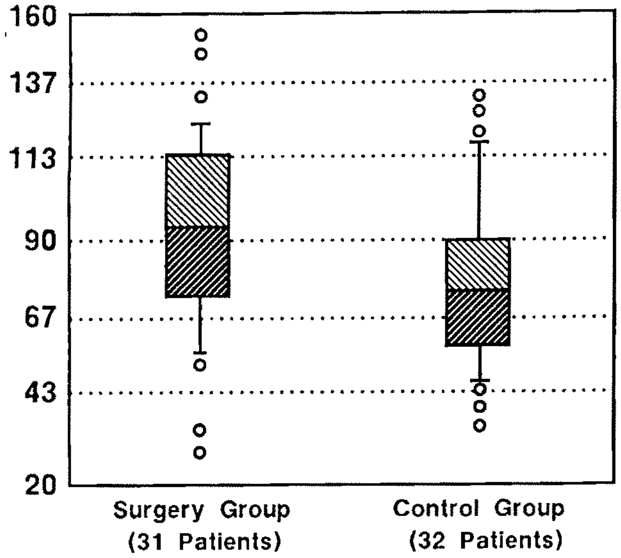
REPRINTS — Itzhak Brook, MD, MSc, PO Box 70412, Chevy Chase, MD 20813-0412.



Levels of serum antibodies to *Prevotella intermedia* in 31 patients who had elective tonsillectomy and 32 controls. Determined by enzyme-linked immunosorbent assay.

patients was close to that of the study group. The criterion for inclusion was the lack of a history of recurrent tonsillitis, gingivitis, or periodontitis. None had any other recent acute or chronic upper respiratory tract infection or any other infectious problems within the past year.

Immunologic Method. Antibody titers were measured by ELISA, which was performed by a modification of the methods of Ebersole et al.[3]

Isolates of the following oral bacteria served as sources of antigens: *A actinomycetemcomitans* strain Y4 serotype b (a clinical strain isolated from a child with juvenile periodontitis), ATCC (American Type Culture Collection) strain 9710 serotype c, and ATCC strain 29523 serotype a; *P intermedia* ATCC strain 25611; and *P gingivalis* strain 381 (a clinical strain isolated from an adult with periodontitis). All of the bacteria were grown in trypticase soy broth medium. The bacteria were killed with 1% phenol and harvested by centrifugation at 10,000 rpm for 30 minutes, washed once with phosphate-buffered saline (PBS; pH 7.00), and centrifuged again for 30 minutes more.

One gram of packed wet sediment of Y4 was suspended in 25 mL of distilled water. For strains 9710, 29523, 381, and 25611, 1 g was suspended in 100 mL distilled water. They were then sonicated for 20 minutes and centrifuged for 30 minutes at 4,000 rpm. The supernatant showed that Y4 contained 46 µg/mL of protein, 29523 contained 13 µg/mL, 9710 contained 10 µg/mL, 25611 contained 12 µg/mL, and 381 contained 2 µg/mL. The supernatants were stored at −20°C. Antigens were diluted in PBS of 0.1 mL supernatant plus 9.9 mL PBS buffer (pH 7.3), and 100 µL per well was used to bind to polystyrene microtiter plates and was incubated at 37°C for 4 hours, after which the antigen-coated plates were stored at 4°C for 1 week.

Enzyme-Linked Immunosorbent Assay. The patient sera were diluted 1:4 with 0.01% gelatin in PBS, and seven fourfold dilutions were prepared; 50 µL of each dilution was pipetted into the coated wells. Incubation followed at 37°C for 1 hour, the wells were washed three times with 0.01% gelatin-PBS; 50 µL of the 1:1,000 diluted alkaline phosphatase conjugated goat anti human serum (Cappel-Organon Teknika Co, West Chester, Pa) was added, and the wells were incubated for 1 hour at 37°C. Three washings, as above, were followed by the addition of 200 µL *p*-nitrophenyl phosphate (1 mg/mL) dissolved in glycine buffer at pH 10.4. The plates were kept at room temperature and the optical densities of the wells were read on a Titer-scan plate reader (Flow Labs, McLean, Va) 60 minutes later at 405 nm. The ELISA units were calculated by measuring the area under the antibody dilution curve.

Statistical Analysis. The median test[4] was used to compare the data set from group A to that from group B. For this purpose the data were transformed to conform to the positive and negative control readings for each batch of 20 data values. The positive control reading was assigned an arbitrary value of 100 units, and the negative control reading the value of zero. Actual data values were then transformed as a straight line adjustment between these two values.

RESULTS

The data sets of groups A and B are displayed in the Figure in the form of box plots.[5] The line across the "center" of each box represents the median of the data set. The upper end of the box represents the 75th percentile of the data, and the lower end the 25th percentile. The box, therefore, is representative of the middle 50% of the data. The upper "whisker" of each box extends to the 90th percentile of the data, and the lower "whisker" to the 10th percentile. Data points beyond these percentiles are represented as small circles.

The median test run on the transformed data for *P intermedia* yielded a p value of .0167. We thus conclude that the data in group A tend to be higher than data from group B for that organism (median 91 versus 72.5). The median test was also used to compare data for other bacteria (*P gingivalis* and *A actinomycetemcomitans*). These comparisons, however, did not indicate any statistical differences, and the antibody levels were below 30 units.

DISCUSSION

This study demonstrates the presence of significantly higher levels of antibodies to *P intermedia* in patients with RNST as compared to controls. In contrast, no increase in titers was found to *P gingivalis*, a known cause of adult periodontitis and other dental infections,[6] or *A actinomycetemcomitans*, known to be associated with localized juvenile periodontitis.[7] The lack of increased titers to these two organisms suggests that neither plays a role in recurrent tonsillitis and that the elevated titers to *P intermedia* are not due to dental or other infection. However, it should be noted that an absence of humoral antibody response to bacteria does not eliminate their pathologic role.

Prevotella intermedia, a member of the black-pigmented *Prevotella* species, is a known oral pathogen.[8] It has been associated with adult periodontitis, but in contrast to *P gingivalis* and *A actinomycetemcomitans*, it has also been isolated as a predominant species in several chronic upper respiratory tract infections.[8] It has been isolated in aspiration pneumonia,[9] lung abscesses,[10] chronic otitis media,[11] chronic sinusitis,[12] peritonsillar and retropharyngeal abscesses,[13] and bacteremia.[14] Although bacterial cultures were not obtained from our patients' tonsils, *P intermedia* is one of the most predominant isolates recovered from cases of recurrently inflamed tonsils and has been recovered from over two thirds of these tonsils.[15] *Prevotella intermedia* and other anaerobic bacteria have also been isolated from the cores of tonsils of children with recurrent GABHS[15] and non-GABHS[16] tonsillitis and peritonsillar abscesses.[13] β-Lactamase–producing strains of *Bacteroides*, *Prevotella* sp, *Porphyromonas* sp, *Fusobacterium* sp, and *Staphylococcus aureus* were isolated from the tonsils of over 74% of children with GABHS recurrent tonsillitis[15,17-19] and from 40% of children with non-GABHS tonsillitis.[16]

The possible role of anaerobic bacteria in the acute inflammatory process in the tonsils is supported by several clinical observations: the recovery of anaerobes as predominant pathogens in tonsillar or retropharyngeal abscesses, in many cases without any aerobic bacteria[13]; their recovery as pathogens in well-established anaerobic infections of the tonsils (Vincent's angina[2]); the increased recovery rate of encapsulated pigmented *Prevotella* and *Porphyromonas* isolates in acutely inflamed tonsils[20]; their isolation from the cores of recurrently inflamed non-GABHS tonsils[16]; and the response to antibiotics in patients with non-GABHS tonsillitis.[21-25]

Several recent studies in which metronidazole was administered to patients with mononucleosis supported the role of anaerobes in tonsillitis.[25] Metronidazole alleviated the clinical symptoms of tonsillar hypertrophy and shortened the duration of fever. Metronidazole has little or no antimicrobial activity against aerobic bacteria and is effective only against anaerobes.[8] A possible mechanism of its action could be suppression of the oral anaerobic bacterial flora that might have contributed to the inflammatory process induced by the Epstein-Barr virus.[25]

Several clinical studies illustrated the efficacy of antimicrobial therapy in reducing fever and other clinical signs and symptoms of patients with non-GABHS tonsillitis.[21-25] It is possible that the improvement in clinical signs was due to the efficacy of these antimicrobials against organisms other than GABHS, including anaerobic bacteria.

Foote and Brook[26] pretreated patients scheduled for elective tonsillectomy with either penicillin or clindamycin. Clindamycin was more effective than penicillin in eradicating the β-lactamase–producing bacterial flora as well as GABHS.

McDonald et al[21] demonstrated a reduction in the severity of symptoms of patients with non-GABHS tonsillitis following the administration of erythromycin. Merenstein and Rogers[22] demonstrated definite improvement in the symptoms of patients with acute non-GABHS tonsillitis following penicillin therapy, as compared to placebo. Putto[23] showed an earlier defervescence following penicillin therapy of children with non-GABHS tonsillitis as compared to patients with viral tonsillitis. Brook[24] demonstrated the superiority of clindamycin over penicillin in the therapy of RNST, in reducing fever and preventing recurrences. Of the two, clindamycin was more effective in relieving the signs and symptoms of acute episodes and in the prevention of recurrences. In a 1-year follow-up period, recurrent tonsillitis occurred in 13 of the 20 patients who received penicillin and in 2 treated with clindamycin ($p < .001$). The superiority of clindamycin may be due to its activity against the aerobic and anaerobic β-lactamase–producing bacteria that were recovered from the majority of the patients.

Elevated antibody levels in patients with RNST support the role of anaerobic bacteria such as *P intermedia* in the infectious process. Elevated antibody levels are an accepted indication of past bacterial infection due to several microorganisms, including GABHS. Further studies are indicated to evaluate whether antibody titers to *P intermedia* decrease following tonsillectomy, whether other aerobic and anaerobic organisms play a role in non-GABHS tonsillitis, and whether antimicrobials directed at the eradication of these other organisms improve the clinical outcome and prevent complications.

ACKNOWLEDGMENTS—The authors acknowledge the technical assistance of Rita Azimvidah, who performed the ELISA test, the laboratory staffs of Alachua General and North Florida Hospitals, and the secretarial support of Ann Grossman, MEd.

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HISTOPATHOLOGY OF OLFACTORY MUCOSA IN KALLMANN'S SYNDROME

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Olfactory mucosa was harvested by intranasal biopsy from a man with Kallmann's syndrome in whom the absence of the olfactory bulbs was documented by magnetic resonance imaging. On electron microscopic examination, several pathologic changes were evident in the olfactory mucosa. First, most olfactory neurons lacked cilia (ie, were morphologically immature). Second, the fila olfactoria had fewer than the normal number of axons, and a large proportion of them were apparently undergoing electron lucent degeneration. Finally, neuromatous collections of axons were seen superficial to the basement membrane in the epithelium. Similar changes have been observed in the mucosa of experimentally bulbectomized rodents. Accordingly, a constellation of pathologic changes — axonal degeneration, neuronal immaturity, and the formation of intraepithelial neuromas — seems to be characteristic of olfactory mucosa that cannot innervate the olfactory bulb in both humans and animals. On the basis of our observations, it is worth investigating the status of the olfactory bulb in other forms of human anosmia in which similar morphological changes are observed in the mucosa, such as persistent posttraumatic anosmia and isolated congenital anosmia.

KEY WORDS — Kallmann's syndrome, olfactory mucosa.

Kallmann's syndrome, or olfactogenital dysplasia, is a heritable condition characterized by the association of anosmia and hypogonadotropic hypogonadism.[1,2] The anosmia can be explained by the absence of the olfactory bulbs and tracts, which has been demonstrated in all reported autopsies of affected individuals,[3] while the hypogonadotropic hypogonadism is due to the lack of normal secretion of luteinizing hormone releasing hormone (LHRH).[4] Recently, evidence has been presented that suggests that LHRH secretion is abnormal in Kallmann's syndrome because the usual migration of LHRH-expressing neurons during development is interrupted.[5] During normal development, LHRH neurons originate in the olfactory placode, migrate along the terminal nerves to enter the brain, and finally reach the hypothalamus and preoptic area.[5,6] However, the examination of the brain and nasal tissue of a fetus of 19 weeks' gestation with a terminal deletion of the X chromosome, which was associated with Kallmann's syndrome in a sibling, demonstrated that LHRH neurons had accumulated on the nasal side of the cribriform plate and were not evident in the brain.[5]

In patients with Kallmann's syndrome, the status of the olfactory epithelium and of the axonal projection from the olfactory neurons is less well understood. In the fetus with the X chromosome deletion described above, olfactory axons apparently ended in a neuromatous tangle at or just above the cribriform plate, as based on the appearance of Nissl-stained sections[5]; the result suggests that the olfactory epithelium had developed to some extent in this case (although the condition of the olfactory epithelium was not directly described) and that the failure of the bulb to form was not due to a total absence of the epithelium and the nerve. On the other hand, olfactory epithelium has not previously been demonstrated in biopsies of the olfactory region from adult patients with Kallmann's syndrome.[7,8]

The purpose of this case study is to report our identification of olfactory epithelium in a man with Kallmann's syndrome and to describe the pathologic changes observed. Our findings have implications for the developmental mechanism underlying the absence of the bulbs and the corresponding anosmia. Moreover, the description of the morphological changes in the olfactory epithelium in this patient, which closely resemble those observed in bulbectomized animals, enhances our understanding of the pathophysiology underlying other forms of anosmia by defining the constellation of histopathologic findings that can be ascribed to the absence of the olfactory bulb in humans.

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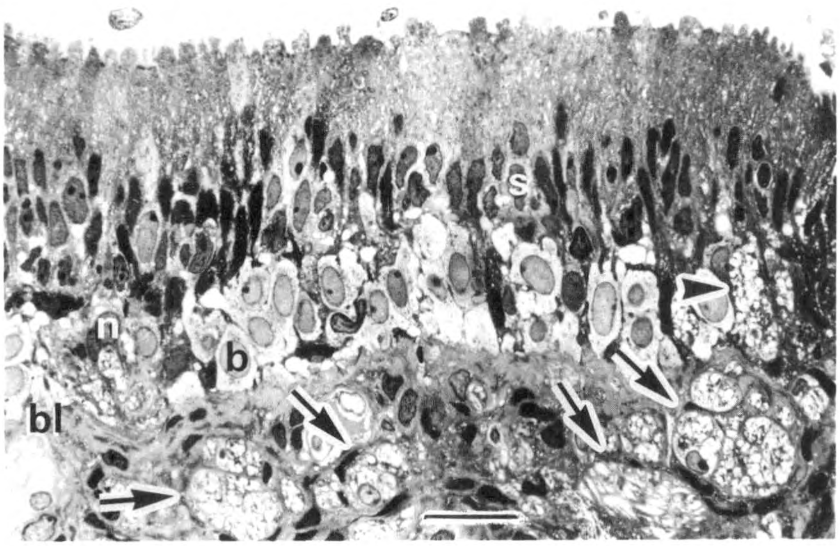

Fig 1. Semithin plastic section of olfactory epithelium from patient with Kallmann's syndrome. Supporting cells (s), neurons (n), and basal cells (b) can be recognized by comparison with electron micrographs of thin sectioned material (Fig 2). Arrows indicate fila olfactoria, all of which are foamy in appearance as consequence of axonal swelling and degeneration noted on electron microscopy (Fig 4). Arrowhead indicates neuromatous collection of axons in epithelium, superficial to basal lamina (bl); note that this intraepithelial neuroma, too, appears foamy, consistent with electron micrographs in Fig 3. Bar — 20 μm.

CASE REPORT

The patient is a 27-year-old man who presented at age 17 with failure to enter puberty and absence of secondary sexual characteristics; in addition he reported that he lacked a sense of smell. By the patient's report his younger brother and maternal uncle also lack a sense of smell; their endocrinologic status is currently unknown. The physical examination findings were normal except for the lack of facial hair, microphallus, and small testes. Serum levels of the gonadotropins were low: follicle-stimulating hormone (FSH) was less than 3 mU/mL (normal 4 to 25), luteinizing hormone (LH) was less than 5 mU/mL (normal 7 to 24), and testosterone was 33 ng/mL (normal 300 to 1,200). Otherwise, the laboratory results were normal, and evaluation included a buccal smear, serum thyroid-stimulating hormone, thyroxine, and prolactin determinations. Radiographs of the sella turcica were also normal. A diagnosis of probable Kallmann's syndrome was made, and the patient was started on a regimen of testosterone enanthate in oil. During the subsequent year, he had a 23-cm growth spurt, a 20-kg weight gain, and the development of secondary sexual characteristics.

He presented at age 26 for analysis and treatment of his infertility, at which time he was referred to the SUNY Olfactory Research Center at Syracuse for further evaluation of his anosmia. At that time his performance on the 10-item Odorant Confusion Matrix (OCM; an odorant identification task[9]) was 8% correct, which is equivalent to random performance. He was unable to identify any of the tested odorants consistently, including stimuli like vinegar and ammonia for which activation of the trigeminal nerve in the nose could normally contribute to the perception of the odorant. He was able to identify sweet, sour, salty, and bitter taste stimulants correctly. Further radiologic evaluation included a computed tomographic examination of the sinuses and pituitary areas, both of which were normal. In addition, magnetic resonance imaging of the vicinity of the cribriform plate was performed in the horizontal, sagittal, and coronal planes, but the olfactory bulbs could not be visualized. Moreover, the olfactory sulci were shallow and poorly developed, as has been described in other patients with Kallmann's syndrome.[10] In contrast to our observations in this case, the bulbs can be visualized with magnetic resonance imaging in patients with normal olfaction at our institution (results not shown). After we obtained informed consent in accordance with our Institutional Review Board–approved protocol, the nasal septal mucosa in the olfactory region was sampled for biopsy from two locations on each side; the techniques used were those originally described by Lovell et al.[11] The biopsy specimens were fixed overnight in 0.6% paraformaldehyde–2% glutaraldehyde in 0.06 mol/L sodium cacodylate buffer with 0.05 mol/L calcium chloride at pH 7.2, rinsed in buffer, postfixed in osmium tetroxide, dehydrated, and embedded in araldite. Semithin plastic sections stained with toluidine blue were evaluated with the light microscope, and thin sections were stained with lead citrate and uranyl acetate and examined with a JEOL JEM 100 CXII electron microscope.

HISTOPATHOLOGIC OBSERVATIONS

In two of the four biopsy specimens (one from each side of the nose) olfactory mucosa could be recognized with the light microscope by the pseudostratified appearance of the epithelium, by the microvillus-capped supporting cells in the epithelium, and by the fila olfactoria (fascicles of the olfactory nerve) in the lamina propria deep to the epithelium (Fig 1). In

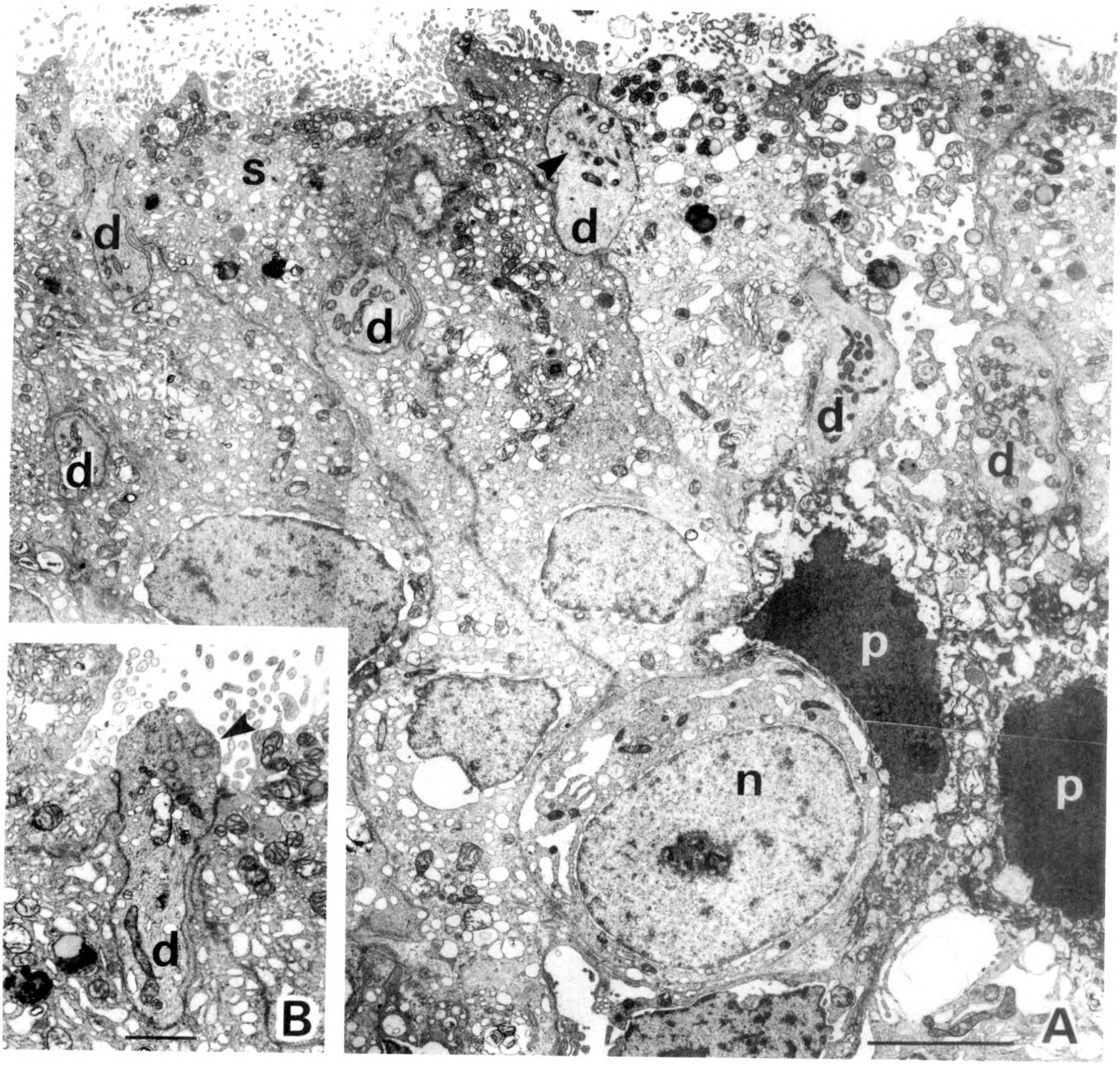

Fig 2. In olfactory epithelium, most neurons are immature. A) This area of epithelium contains neurons (n), some pyknotic cells that may be neurons (p), and microvillus-capped supporting cells (s) lining apical surface. Apical dendrites (d) are identifiable at epithelial surface and deeper in epithelium by their round-to-oblong shape and clusters of mitochondria, filaments, and migrating centrioles (arrowhead). Bar — 4 µm. B) Example of dendritic knob, protruding at surface of epithelium, which lacks cilia. Note that centrioles (arrowhead) tend to be grouped toward center of knob, and have not yet formed basal bodies. Both of these features are indicative of neuronal immaturity.[13-15] Bar — 1 µm.

comparison to mucosal biopsies from adults with normal olfaction[12] (also Schwob and Szumowski, unpublished observations), the mucosa is thinner, being only three or four cell layers thick, and the fila olfactoria appear vacuolated and pale. Nonetheless, olfactory neurons are not rare, and they and their processes can be seen with the electron microscope. For example, there are multiple dendrites in the field illustrated in Fig 2A, which can be identified by their size, the clustering of mitochondria, and/or the inclusion of centrioles. The presence of dendrites indicates that neurons are numerous in the vicinity, and at least one neuronal soma is seen in this section. However, the dendritic knobs at the surface of the epithelium do not have cilia (Fig 2B[13-15]); hence most, if not all, of the olfactory neurons are morphologically immature. Pyknotic cells are also present in the middle layers of the epithelium and may correspond to degenerating neurons (Fig 2A).

In addition to the finding that most neurons are immature, the population of olfactory axons is also distinctly abnormal in several respects. First, the number of olfactory axons in any given fascicle of the

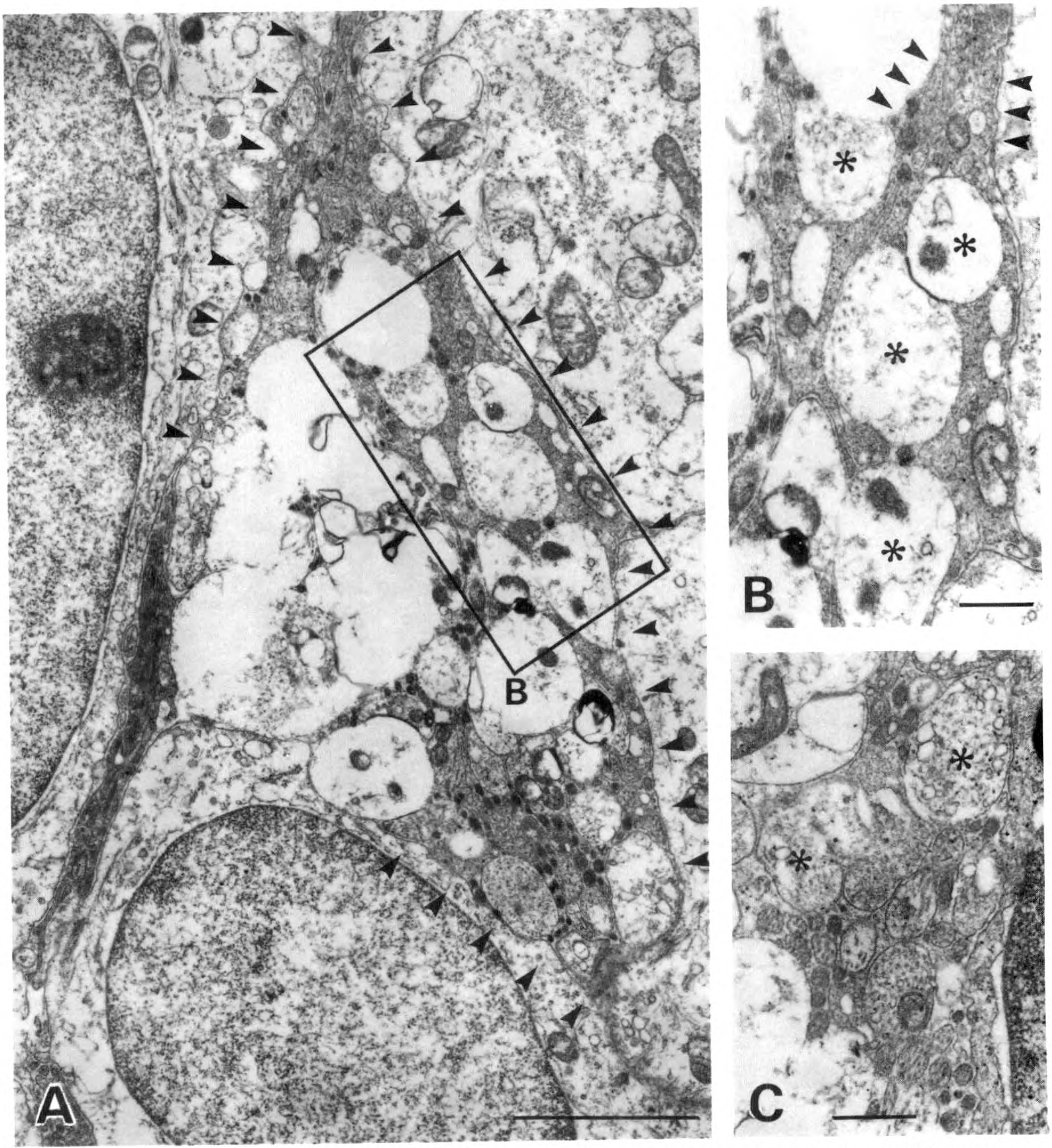

Fig 3. Abnormal collections of axons can be found in epithelium above basement membrane. A) This neuroma (outlined by arrowheads) is situated among cells of olfactory epithelium. Bar — 2 μm. B) Higher magnification of boxed area in A. Bar — 0.5 μm. B,C) Neuromas contain both degenerating axons (asterisks) and others that are more normal in appearance (arrowheads in B). Bar — 0.5 μm.

olfactory nerve is substantially less than normal. For example, in many instances the investing loops of the Schwann cells enclose only one or a few axons instead of the tens or hundreds that would normally be present in a single trough. Second, collections of olfactory axons are found superficial to the basement membrane in the olfactory epithelium (Fig 3). Third, many of the axons in both the fila olfactoria and the intraepithelial neuromas are swollen, their cytoplasms are pale and often contain phagosomes, and the surface membranes are often ruptured and fragmented (Fig 4). Other axons are smaller in diameter and contain cytoplasm that is of normal density, suggesting that the changes are not likely to be artifactual. In experimental animals, similar changes characterize axons undergoing electron-lucent degeneration distal to a transection of the olfactory nerve.[16]

DISCUSSION

In our patient with Kallmann's syndrome, olfactory epithelium is demonstrably present, despite the absence of olfactory bulbs. However, the mucosa is abnormal in appearance: it contains mainly immature

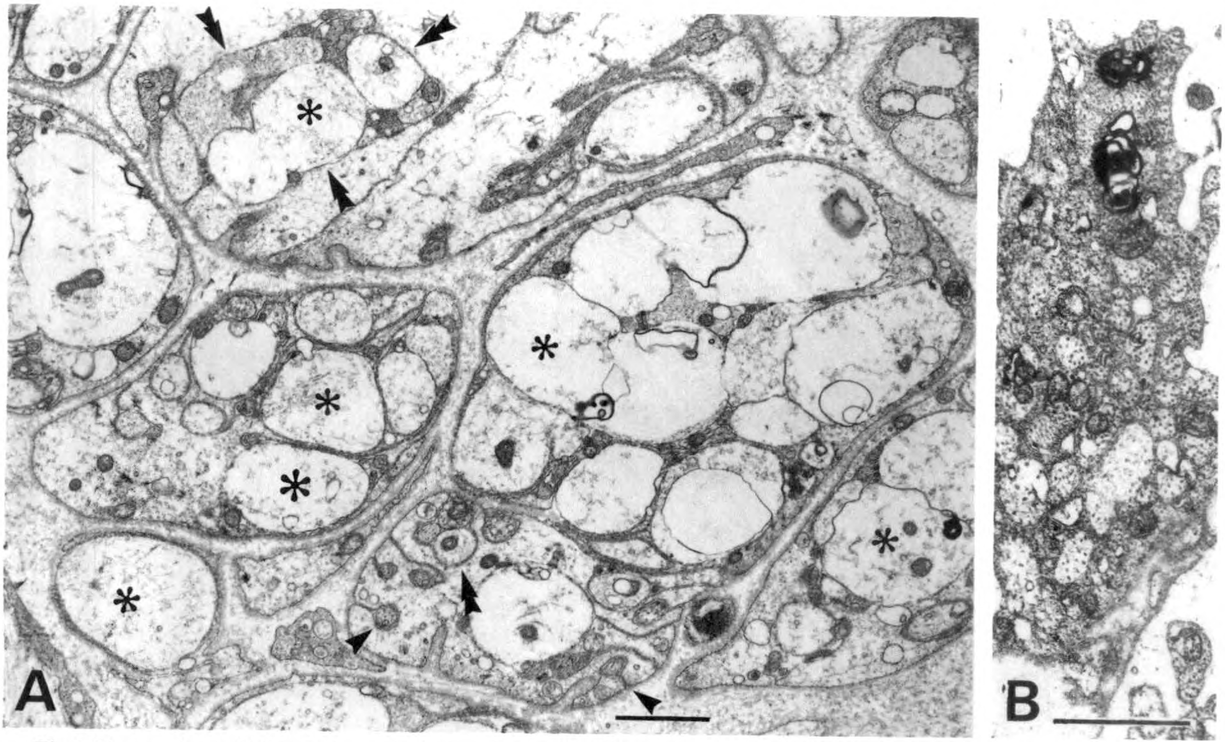

Fig 4. Bars — 1 μm. A) Lamina propria from patient with Kallmann's syndrome. Most axons in this particular fascicle of olfactory nerve and in other fila olfactoria in lamina propria are degenerating. Examples of axons undergoing electron-lucent degeneration are indicated by asterisks. In addition, number of axons is less than normal. Many Schwann cell troughs contain single axon (single arrowheads), and others have only few (double arrowheads), in contrast to 20 to 100 axons that are typical of normosmic individuals (see B). B) Example of axonal bundle that is harvested from lamina propria of normosmic individual. Entire bundle, numbering greater than 50 axons, is ensheathed within single Schwann cell trough. In general, axons are small and relatively uniform in diameter, without much evidence of electron-lucent degeneration.

neurons, ie, those lacking ciliated olfactory knobs; there is disordered axonal growth, evidenced by the intraepithelial neuromas; there is widespread degeneration of olfactory axons, suggesting that neuronal turnover is rapid; there are fewer olfactory axons in the fila olfactoria than normal. The previous inability to demonstrate olfactory epithelium in patients with Kallmann's syndrome[7,8] may simply represent a sampling error. Alternatively, the decrease in the number of axons in the fila olfactoria may be an indication that the population of neuronal stem cells in the epithelium is being exhausted, as has been previously suggested[7]; indeed, the two previous cases that were submitted to biopsy and reported in the literature were in older patients[7,8] (35 and 52 years, respectively). The testosterone therapy is unlikely to explain any difference between this individual and other Kallmann's patients who underwent mucosal biopsy, as they had also been treated with testosterone at some time prior to biopsy.[7,8] It is interesting to note that olfactory neuroblastoma has been reported in a patient with Kallmann's syndrome.[17]

The constellation of findings in this case resembles the changes in the olfactory mucosa observed in experimental animals many months after olfactory bulb ablation.[13,14] After bulbectomy, the rate of neurogenesis remains chronically elevated as compared to normal, yet olfactory neurons born in the absence of the bulb die prematurely as compared to the life span in the normal epithelium.[13,14] As a reflection of the accelerated neuronal turnover in the mucosa, axons that are undergoing electron-lucent axonal degeneration are very prevalent in the fila olfactoria, even at very long survivals after bulbectomy, as compared to normal.[13,14] Moreover, most of the neurons in the epithelium of bulbectomized rats are immature by both ultrastructural and immunohistochemical criteria[13,14,18,19]; this finding suggests that they are dying before having a chance to fully differentiate. Finally, intraepithelial neuromas can form after bulb ablation in animals as well.[20,21]

The significance of our findings is twofold. First, the absence of the bulb, which is typical of Kallmann's syndrome, is not a consequence of a failure of the olfactory epithelium to form. Rather, the more likely cause is the failure of the growing olfactory axons to contact the prosencephalic vesicle, which, from other evidence, seems to be responsible for inducing the formation of the olfactory bulb.[22] The failure-of-induction hypothesis is consistent with the demonstration that the putative X-linked gene responsible for Kallmann's syndrome encodes an adhesionlike protein[23,24] whose absence or abnormality might interfere with both cell migration and axon outgrowth. (It is worth noting that this patient's family history is consistent with the X-linked form of Kallmann's syndrome.) Second, the results allow us to define a constellation of histopathologic changes that occur as a consequence of the absence of the olfactory bulb in humans. The demonstration that a similar constellation of findings can be seen in other forms of anosmia — for example, in isolated congenital anosmia[25,26] and persistent anosmia following head trauma[27] (also Schwob and Szumowski, unpublished observations) — suggests that the connection between the olfactory epithelium and the central nervous system has been interrupted in these disorders as well. According to this hypothesis, the persistent anosmia following head trauma could result either from damage to the olfactory bulb or from the inability of olfactory axons to traverse the cribriform plate and contact the bulb. Consequently, the investigation of the anatomic status of the bulb, for example by magnetic resonance imaging, is a potentially fruitful avenue for enhancing our understanding of the olfactory disorders, or others in which similar histopathologic changes occur, and perhaps for suggesting potential means of treatment.

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EFFECT OF OXYMETAZOLINE ON NASAL AND SINUS MUCOSAL BLOOD FLOW IN THE RABBIT AS MEASURED WITH LASER-DOPPLER FLOWMETRY

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The effect of topical oxymetazoline hydrochloride on the blood flow of the nasal and sinus mucosa of the rabbit was measured by laser-Doppler flowmetry. Oxymetazoline, the active component in clinically used nose drops, induced a dose-dependent decrease of the nasal mucosal blood flow. This effect has previously been shown in humans and suggests the presence of α2-adrenoceptors in the nasal mucosa of the rabbit. Doses of oxymetazoline used clinically in humans induced a 50% reduction of blood flow in rabbits. Rhythmic variations in blood flow were seen in 30% of the rabbits after administration of oxymetazoline. Additionally, oxymetazoline induced a dose-dependent decrease of the mucosal blood flow in the maxillary sinus when the drug was applied in the nose. A vasoconstricting effect of oxymetazoline on the arteries penetrating the maxillary sinus ostium is a possible explanation. This can have positive as well as negative consequences on acute sinus infections.

KEY WORDS — blood flow, flowmetry, imidazoles, microcirculation, nasal sinus mucosa, pharmacology, vasomotion.

INTRODUCTION

Topical nasal decongestants fall into two categories, namely imidazoline derivatives (eg, oxymetazoline hydrochloride, xylometazoline hydrochloride) and sympathomimetic amines (eg, phenylephrine hydrochloride). The congestion of the nasal mucosa in different types of acute rhinitis is due to vasodilation of the sinuses and to interstitial edema. Nose drops act by constricting the blood vessels in the nasal mucosa. Imidazoline derivatives are selective α2-adrenoceptor agonists, while sympathomimetic amines act on the α1-adrenoceptors.[1] This different mode of action might be important, as the sinusoids in the human nasal mucosa are regulated by α1- as well as by α2-adrenoceptors, while the resistance vessels are not significantly affected by α1-adrenoceptor agonists as measured with the xenon 133 washout technique.[2,3] Treatment with nasal decongestants of the imidazoline type leads to a decreased blood flow[4-6] as well as decongestion in humans, where the duration of these effects is on the order of 6 hours.[7]

Changes in local blood flow cause warmth, redness, and swelling, which are cardinal signs of inflammation. Blood flow changes are important during infectious and other inflammatory conditions. Drugs interfering with blood flow modify the local defense reaction and potentially counteract an adequate inflammatory response.

The aims of this investigation were to evaluate 1) the effect of nasally applied oxymetazoline on the nasal mucosal blood flow of the rabbit, and to find a dose that equals the effects on blood flow found in humans with clinically recommended doses, as a basis for further study, and 2) how the sinus mucosal blood flow is affected by nasally applied oxymetazoline.

MATERIAL AND METHODS

Animal Model. Male New Zealand White rabbits (1.8 to 2.1 kg) were sedated with fentanyl citrate–fluanisone (Hypnorm, 100 μL/kg) and anesthetized intravenously with 20% urethane (1.5 g/kg body weight). The body temperature was maintained at 37.5°C by a heating pad controlled by a rectal thermostat. The animals were breathing spontaneously at a rate of 50 to 60 breaths per minute and were tracheotomized to facilitate breathing. After catheterization of the femoral artery and vein, the nasal mucosa of the septum was prepared for laser-Doppler

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From the La Jolla Institute for Experimental Medicine (Åkerlund, Arfors) and the Department of Applied Mechanics and Engineering Sciences, University of California–San Diego (Bende, Intaglietta), La Jolla, California. This investigation was supported by the Public Health Service Fogarty International Research Fellowship (1 FO5 TWO4483-01) and US Public Health Service grant HLIB 12493, United States; and the Swedish Society of Medicine, the Swedish Medical Research Council (B91-17F-9379-01), the Nanna and Albert Skantze Memorial Foundation, the Tore Nilsson Foundation for Medical Research, and the County Council of Skaraborg, Sweden. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals,* and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the La Jolla Institute for Experimental Medicine, La Jolla, California.

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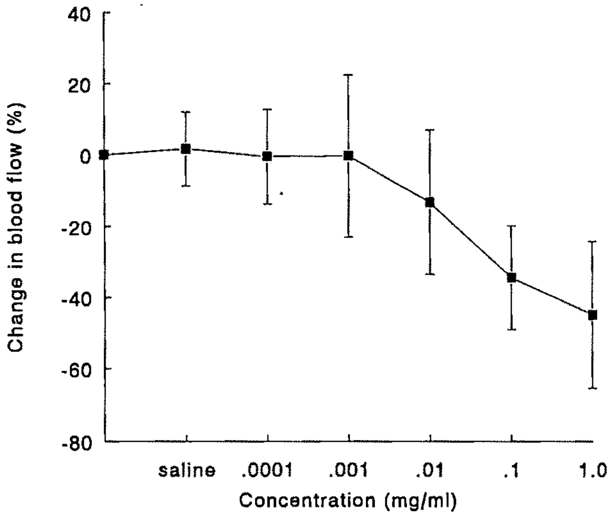


Fig 1. Nasally applied oxymetazoline at different concentrations caused dose-dependent reduction of nasal mucosal blood flow as measured with laser-Doppler flowmetry. (Mean change ± SD, n = 7).

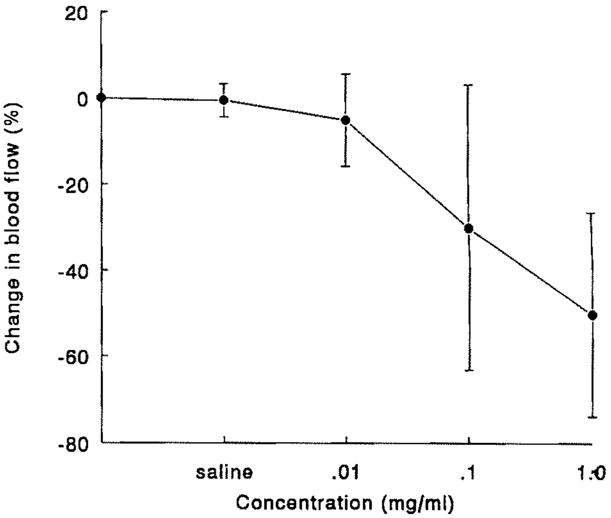


Fig 2. Nasally applied oxymetazoline at different concentrations caused dose-dependent reduction of sinus mucosal blood flow as measured with laser-Doppler flowmetry. (Mean change ± SD, n = 6).

flowmetry (LDF) in vivo. To do this a skin flap of the nasal dorsum was surgically dissected to reveal the maxillary sinuses. Bone over the sinus cavity was carefully removed by dissection, and the sinus cavity was opened by an incision in the mucosa. Either of the following preparations was then performed.

1. In seven animals the sinus medial wall was removed and blood flow on the septal mucosa investigated. Saline and different concentrations of oxymetazoline solution were applied topically on the mucosa close to the LDF probe.

2. In six animals the blood flow in the mucosa of the medial wall of the superior compartment of the maxillary sinus was investigated. Saline and oxymetazoline were applied topically into the nose by a syringe and a small tube (PE-160, outside diameter 1.57 mm), 35 mm from the opening of the nose. In this way the tip of the tube was applied close to the ostium of the maxillary sinus. The location of the tip was confirmed after euthanasia.

After completing the preparation, with the head of the rabbit secured with an Olympic Vac-pac (Olympic Medical, Seattle, Wash), the LDF probe was directed against the mucosa. The probe was fixed with a micromanipulator (Zeiss) and adjusted to be about 1 mm from the mucosa. The probe was not placed in direct contact with the tissue, to avoid introducing perturbation due to compression artifacts. Proper instrument function was monitored by ensuring that the combination of input and reflected light was within the instrumentation requirement, which was indicated by a light signal, which in this instrument is used to monitor this condition via a special circuitry.

The output signal of the LDF (Periflux PF 1d, Perimed, Sweden) was recorded continuously during the experiments. The limit frequency was set to 4 kHz, the time constant to 3 seconds, and the gain control at x10. The output signal was continuously registered after analog to digital conversion in a computer data acquisition system with a sampling rate of 4/s.

Arterial pressure was monitored continuously, and heart rate was determined from the pressure tracing. Arterial blood gases were measured intermittently during the experiments and were throughout within the physiologic range for the rabbit (PO_2 over 9.8 kPa and PCO_2 under 5.9 kPa).

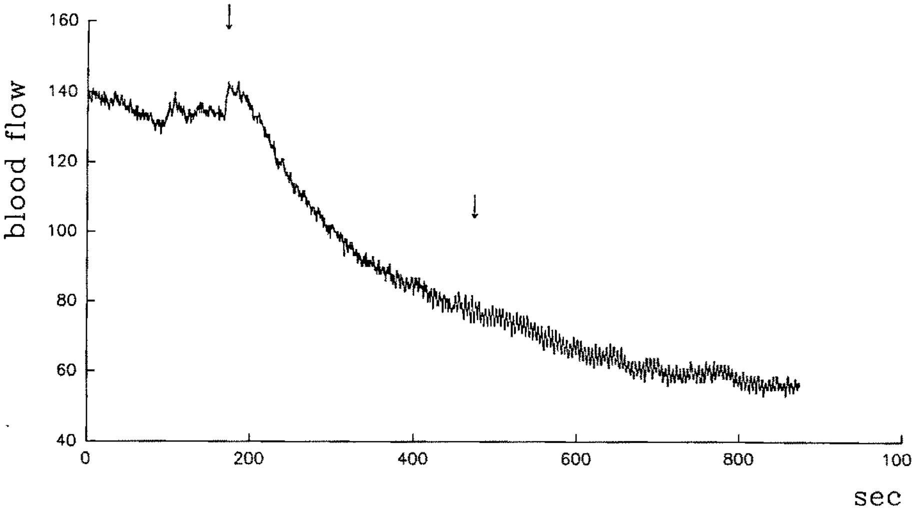
Drugs. Saline and oxymetazoline (0.0001 to 1 mg/mL) were administered cumulatively, 0.1 mL at a time. On each successive administration the dosage was increased by a factor of 10. After each administration the blood flow decreased, and it achieved stable value when 4 to 5 minutes had elapsed. At this time, blood flow was measured during a period of 2 minutes. All drugs were kept refrigerated and were warmed to room temperature (20°C) before use.

Statistical Analysis. Results were expressed as means of relative changes ± SD. Student's paired *t* test was used to test the effect of saline compared to initial values. Analysis of variance was done by Duncan's multiple range test to test the effect of oxymetazoline. Only p values less than .05 were considered statistically significant.

RESULTS

Topical saline did not induce any significant changes of the mucosal blood flow as measured by

Fig 3. Part of laser-Doppler registration as recorded by microprocessor. First arrow indicates when oxymetazoline was administered. Second arrow shows periodic flow changes, possibly attributed to vasomotion (14 cycles per minute), observed in 4 of 13 experiments. Blood flow is in arbitrary units.



LDF (Figs 1 and 2). The effect of oxymetazoline on blood flow was calculated as a percentage of the LDF output registered during application of saline.

Oxymetazoline applied topically in the nose induced a dose-dependent decrease of the nasal mucosal blood flow (Fig 1). Oxymetazoline reduced the blood flow significantly in the concentrations 0.1 and 1.0 mg/mL when compared to saline ($p < .05$ and $p < .01$). The difference in effect between the oxymetazoline concentrations 0.01 and 0.1 mg/mL was statistically significant ($p < .05$). A dose-response effect was found also on the blood flow in the maxillary sinus mucosa after nasal administration of oxymetazoline (Fig 2). Here oxymetazoline reduced blood flow significantly in the concentrations 0.1 and 1.0 mg/mL when compared to saline ($p < .01$). The difference in effect between the oxymetazoline concentrations 0.01 mg/mL and 0.1 mg/mL was statistically significant ($p < .05$). We found that 0.1 mL of oxymetazoline in a concentration of 1 mg/mL applied on the nasal mucosa induced a 50% reduction of the nasal mucosal blood flow, as well as of the sinus mucosal blood flow.

Before and after the administration of saline, there were irregular flow fluctuations that were on the order of plus or minus 3% of the signal. After application of oxymetazoline, however, the output was more stable, with less incidence of irregular variations. In 4 of 13 registrations, regular flow variations of 9 to 14 cycles per minute were observed after oxymetazoline was given (Fig 3).

Blood pressure and heart rate were not affected by the administration of topical oxymetazoline in the present dosages. Urethane, administered in small doses when needed for adequate anesthesia, caused an immediate decrease in blood pressure for about half a minute. The mucosal blood flow decreased and then increased during a shorter period (10 to 15 seconds). These variations seemed to be correlated with the depth of the anesthesia.

The LDF signal obtained during postmortem registrations 5 minutes after euthanasia was 4% to 6% of the LDF output obtained at the start of the experiment.

DISCUSSION

The LDF is a noninvasive instrument for evaluating microvascular flow. Light reflected by the moving cells undergoes a frequency shift according to the Doppler principle, while the light scattered into the static tissue remains at its original frequency. The frequency shift is recorded and processed to yield an output voltage that is proportional to the flux of cells, ie, the product of the number of moving cells and their mean velocity.[8] One problem with the LDF is to estimate the penetration depth of the laser light beam in different tissues. When measuring tissues less than 1 mm, we may assume that blood flow in the whole thickness of the tissue is measured. The thickness of the nasal and sinus mucosa of the rabbit is about 0.1 mm.[9]

Oxymetazoline appears to induce rhythmic variations in blood flow in the nasal mucosa with cycles of 9 to 14 per minute. Such variations in the laser-Doppler registrations have been described in human skin with cycles of 5 to 10 per minute[10] and 4 to 9 per minute,[11] and in the same range in rat testis.[12] This phenomenon could be due to vasomotion, which might be induced by oxymetazoline. This might be an item for further study.

Oxymetazoline applied topically induces a dose-dependent decrease of the nasal mucosal blood flow in humans as measured with the xenon 133 washout technique and with LDF.[4,6] The concentration of oxymetazoline (0.5 mg/mL) that is clinically used in nose drops induces a 50% to 60% reduction of the mucosal blood flow. In the rabbit, oxymetazoline nose drops (0.1 mg/mL) applied on the sinus mucosa have been found to decrease the sinus mucosal blood flow more than 50% as measured by the microsphere technique, by the rubidium 86 chloride technique, and by fluorescein flowmetry.[13] Similar effects have been found by LDF in the nasal mucosa of the pig with intraarterially administered oxymetazoline.[14] Our results are in accordance with these previous findings, and α2-adrenoceptors seem to be present in the vascular bed of the nasal mucosa in the rabbit.

The reducing effect on blood flow after topical application of 0.1 mL oxymetazoline in a concentration of 1 mg/mL on the rabbit nasal mucosa corresponds to that seen in clinical use in humans. The animals in all groups reacted to the application of saline with little variability relative to the control baseline. The reactivity to the different concentrations of oxymetazoline presented a greater variability, primarily because of the different reactivity between animals.

The arterial vascular supply for the mucosa of the sinus in the rabbit enters by vessels leading through the maxillary ostium to radiate inside the sinus.[9] The present investigation showed that oxymetazoline applied nasally induces a decreased blood flow in the maxillary sinus, likely by vasoconstriction of these vessels. The arterial blood supply to the mucosa of the maxillary sinus is presumed to enter through the ostium in humans,[15] and nasally applied nose drops can be expected to have a vasoconstricting effect on the sinus mucosa in humans, too. In the nasal mucosa a decreased blood flow is accompanied by decongestion after application of oxymetazoline. If the same is true for the maxillary sinus mucosa, symptoms due to mucosal swelling in the sinus such as aching and pressure can be relieved by nose drops. Additionally, decongestion will contribute to keeping the ostium open to allow proper ventilation of the sinus cavity and prevent a low P_{O2}, which can predispose to development of purulent sinusitis by nonfavorable effects on the local mucosal defenses.[16,17] On the other hand, in the case of a totally obstructed ostium and a sealed-off sinus with empyema, a pharmacologic intervention that decreases the sinus mucosal blood flow may additionally impair oxygenation and access for immunologically potent cells from the circulation,[18] as found experimentally in the rabbit.[19]

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LASER-DOPPLER MEASUREMENTS AND ELECTROCOCHLEOGRAPHY DURING ISCHEMIA OF THE GUINEA PIG COCHLEA: IMPLICATIONS FOR HEARING PRESERVATION IN ACOUSTIC NEUROMA SURGERY

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Interruption of cochlear blood flow has been implicated as one of the causes of the sensorineural hearing loss that may occur during acoustic neuroma surgery. With the guinea pig as an animal model for cerebellopontine angle surgery, laser-Doppler measurements were used to estimate the cochlear blood flow changes caused by compression of the eighth nerve complex. With compression, the laser-Doppler measurements decreased abruptly; somewhat later, the electrocochleographic potentials declined. When compression was released, laser-Doppler measurements usually returned immediately, followed later by return of the electrical potentials. Some of these potentials, including the compound action potential of the auditory nerve, often became transiently larger than their precompression values. Interposing bone between the laser-Doppler probe and the otic capsule, so that the total bone thickness approximated the thickness of the human otic capsule, decreased the laser-Doppler measurement, but changes caused by compression were still apparent. Thus, although the human otic capsule is much thicker than the guinea pig capsule, it may still be possible to make laser-Doppler estimates of human cochlear blood flow. Laser-Doppler monitoring during acoustic neuroma surgery may be beneficial, because it could give earlier warning of ischemia than is currently available from electrocochleographic monitoring, thereby enabling earlier corrective action. Electrocochleography complements laser-Doppler measurements by indicating the physiologic state of the cochlea.

KEY WORDS — acoustic neuroma, blood flow velocity, cochlea, Doppler velocimetry, electrocochleography, laser velocimetry.

INTRODUCTION

A common (about 60%) form of morbidity associated with acoustic neuroma surgery is profound hearing loss. The chance of saving "useful" hearing is roughly inversely related to tumor size, yet even with the smallest detectable tumors a quarter of the patients will not retain "useful" hearing[1] after surgery from a suboccipital posterior fossa approach to the cerebellopontine angle. It has been widely speculated that ischemia during or immediately following the surgery is responsible for many of these cases of hearing loss,[1-6] mainly because major injury to the auditory nerve or cochlea is not observed during surgery. The principal arterial blood supply to the cochlea is via the internal auditory artery, which travels with the auditory, vestibular, and facial nerves in the internal auditory canal. Intraoperative electrocochleography (ECochG) has provided indirect evidence for cochlear ischemia accounting for some cases of intraoperative hearing loss.[7] A frequent ECochG observation has been the abrupt loss of the compound action potential of the auditory nerve (N1), often associated with decreases in cochlear microphonic potentials (CM). A similar pattern of changes in N1 and CM occurs with cochlear ischemia in animal studies.[8]

Monitoring cochlear blood flow during surgery has at least two potential benefits. First, it may provide direct evidence that cochlear ischemia is related to the hearing loss, and second, it may improve the outcome by providing earlier warning (than ECochG) that the cochlea is ischemic, before irreversible hearing loss sets in. Evoked potentials may not be the best way to monitor ischemia, because the inner ear must be deprived of its blood supply long enough for metabolic reserves to drop to the point at which electrophysiologic activity begins to fail.[8] Observations in one case suggest that the time from interruption of the internal auditory artery to changes in the ECochG is between 20 and 50 seconds.[9]

Several techniques have been used to measure cochlear blood flow in experimental animals, including direct visualization of the vascular bed,[10-12] microspheres,[13,14] intracochlear partial pressure of oxygen,[15,16] electrical impedance plethysmography,[17] and laser-Doppler (LD) measurements.[18] Of these methods, only LD measurements have the advantage of being noninvasive to the cochlea and

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From the Neurology Service, Massachusetts General Hospital (Levine), the Departments of Otolaryngology (all authors) and Physiology (Brown), Harvard Medical School, and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary (Levine, Brown), Boston, Massachusetts. Supported by National Institutes of Health grants DC00119 and DC01089. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Massachusetts Eye and Ear Infirmary.

thereby applicable to humans. Changes in LD measurements and more direct measures of cochlear blood flow are highly correlated when the flow is manipulated by several means.[19] No study, however, has monitored LD measurements and electrophysiology simultaneously in the same preparation. Thus, questions such as whether electrophysiologic function can be sustained by the residual flow that remains with eighth nerve compression[20] are unanswered. To address such issues and explore the feasibility of monitoring LD measurements during acoustic neuroma surgery, we have carried out a series of experiments using a guinea pig model for human cerebellopontine angle surgery.

METHODS

Eighty albino or pigmented guinea pigs weighing between 440 and 700 g were anesthetized with urethane (1,500 mg/kg) and Innovar-Vet (0.25 mL/kg), a tracheotomy tube was inserted, the bulla was opened through ventral and postauricular approaches, and a fine silver wire (insulated except at its tip) was placed on the round window to serve as the active electrode for ECochG, with an electrode in the neck muscles serving as the reference. The body temperature was maintained between 36°C and 38°C throughout the experiment.

Laser-Doppler measurements were made on six of these guinea pigs. In one of these animals on the side opposite the exposed cochlea, the carotid artery was cannulated and blood pressure was measured continuously with a calibrated transducer. To make the LD measurements, a TSI (now Vasamedics Inc) LaserFlo blood perfusion monitor (BPM 403A) system was used with a 0.1-second averaging time. The tip of its 0.8-mm needle probe, housing both emitter and collector optical fibers, was positioned in the middle ear against the otic capsule over the spiral ligament of the basal turn (estimated to be at the 16-kHz region of the cochlea) or of the second turn (estimated to be at the 4-kHz region). The angle of the probe was adjusted to obtain maximum LD flow readings. The middle ear mucosa was not removed. In the LD measurement technique,[21-24] a laser beam ("typically 780 nm" for the BPM 403A) leaves the emitter fiber and is directed toward the tissue of interest. The laser light is scattered by the tissues, so that only a small proportion of the light returns to the collector fiber. The moving tissues (red blood cells) cause a Doppler shift in the light that is related to their velocities. There is a spectrum of Doppler shifts of the light returning to the photodetector, thought to be due to several factors, including the number of times a photon is scattered by moving red blood cells, different red blood cell velocities, and different angles of incidence between the photon and the moving red blood cells. Some of this Doppler-shifted light returns to the collector fiber of the LD probe and is heterodyned with the light that has not been Doppler-shifted but has been scattered back to the collector fiber by static tissue. What results is a spectrum of "beat" frequencies representing the difference frequencies between the light collected from static tissue and moving tissue. Signal processing by the BPM 403A obtains the mean difference frequency, which is proportional to the mean velocity of the moving red blood cells (velocity output channel of the BPM 403A, which we shall refer to as LD velocity). The amplitude of the difference frequency signal is thought to be proportional to the number of moving red blood cells present in the sample volume of tissue (volume output channel of the BPM 403A, which we shall refer to as LD number). The product of the LD velocity (millimeters per second) and LD number (number of red blood cells per cubic millimeter) is the LD flux, expressed as ([number of red blood cells)/(seconds x square millimeters]) and appears at the output channel of the BPM 403A labeled "flow." Studies of the BPM 403A have shown that LD flux is linearly related to flow both in vitro and in vivo, but LD flux is reproducible only in vitro.[25] The lack of reproducibility in vivo is a property of the LD technique and not the BPM 403A. It has been attributed to biologic factors such as variations in the hematocrit of the tissue within the field of the laser beam.[25] Although we do not know the penetration depth of laser light in our preparation, estimates from skin and renal tissue indicate that the laser light penetrates to a depth of about 1 mm.[22,26]

After removal of the pinna, an acoustic system consisting of a ½-in condenser ear phone driver was fit to the external auditory canal. Clicks (generated by a 100-microsecond electrical pulse) were presented to the ear continuously at a rate of 10/s and at a level about 20 to 50 dB above threshold (as determined by visual inspection). Averaging of the round window recordings was done continuously throughout each experiment. Generally, after 16 or 32 click presentations an averaged round window response was stored on disk; at the time the waveform was stored on disk the LD flux, LD number, and LD velocity were digitized and stored as well, along with the time interval over which the waveform was obtained. For off-line reanalysis, analog signals of the round window recordings, LD measurements, time code generator, stimulus marker, and voice were recorded on tape.

Through a suboccipital approach to the cerebello-

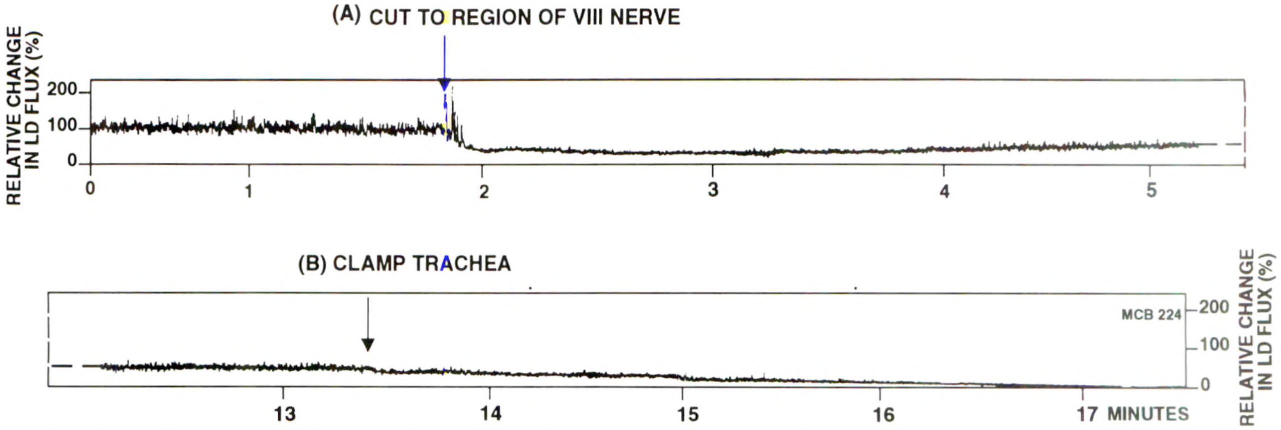

Fig 1. Strip chart recording of laser-Doppler (LD) flux from cochlea of guinea pig. Cut to region of eighth nerve, which presumably severed internal auditory artery, abruptly decreased LD flux by about 50%, and damped its high-frequency fluctuations. High-frequency fluctuations appear as "noise" on top of steady baseline of LD flux, but likely represent changes in LD flux that are related to cardiac cycle.[26] After trachea was clamped to painlessly kill guinea pig, LD flux slowly decreased to zero. Ordinate in this and subsequent Figures is in percent relative to level at beginning of trace.

pontine angle, the cochlear nucleus was exposed by either retracting or aspirating the cerebellum. Landmarks of the cochlear nucleus and adjacent temporal bone were used to direct a 125-μm-diameter wire on a micromanipulator into the eighth nerve complex. In many of these experiments the wire was cemented in place and later verified by gross dissection to be in the auditory division of the eighth nerve. In the LD experiments a micromanipulator-held wire (two experiments), a micromanipulator-held blunt probe (three experiments), or a hand-held blunt probe (one experiment) was used to compress the dorsal surface of the cochlear nucleus.

A calibrated microscope was used to measure otic capsule thickness from five human and two guinea pig cochleas that had been fixed in buffered formalin, decalcified with trichloroacetic acid, dehydrated, embedded in celloidin, sectioned at 20-μm thickness, and stained with hematoxylin and eosin.

RESULTS

Preliminary observations were made from 74 guinea pigs in which continuous ECochG recordings were made while a wire probe was passed through the cochlear nucleus–eighth nerve complex. As the wire probe was advanced dorsoventrally through the cochlear nucleus, there was no change in N1. In 28 of these preparations, however, when the electrode contacted the dorsal surface of the auditory nerve (as confirmed later by dissection), N1 decreased over about 30 seconds. This decline was reversible; with small movements (about 200 μm) of the wire away from the nerve, N1 recovered over about a minute. When the wire was placed rostral or caudal to the auditory nerve, N1 did not change. However, by using a blunt probe, a decline in N1 could also be caused by dorsoventral compression applied to the cochlear nucleus that overlies the eighth nerve complex.

To elucidate the role of cochlear blood flow in these phenomena, we made simultaneous electrophysiologic and LD measurements from the otic capsule of the middle ear in six guinea pigs. In three

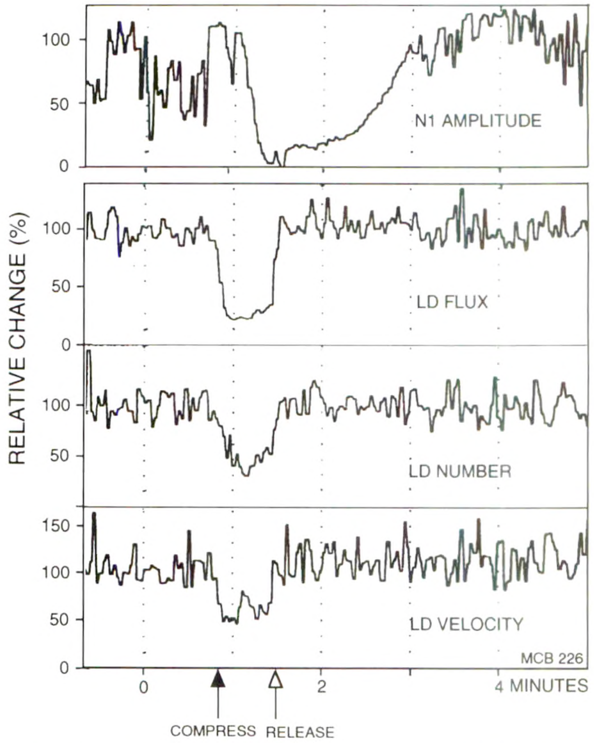

Fig 2. Effect of eighth nerve complex compression on N1 and laser-Doppler (LD) measurements. Laser-Doppler flux, number, and velocity were abruptly decreased by compression and abruptly recovered on release, whereas effects on N1 lagged behind. Some fluctuations in N1 amplitude are due to masking from ambient room sounds. Compression was applied to cochlear nucleus with blunt micromanipulator-held probe.

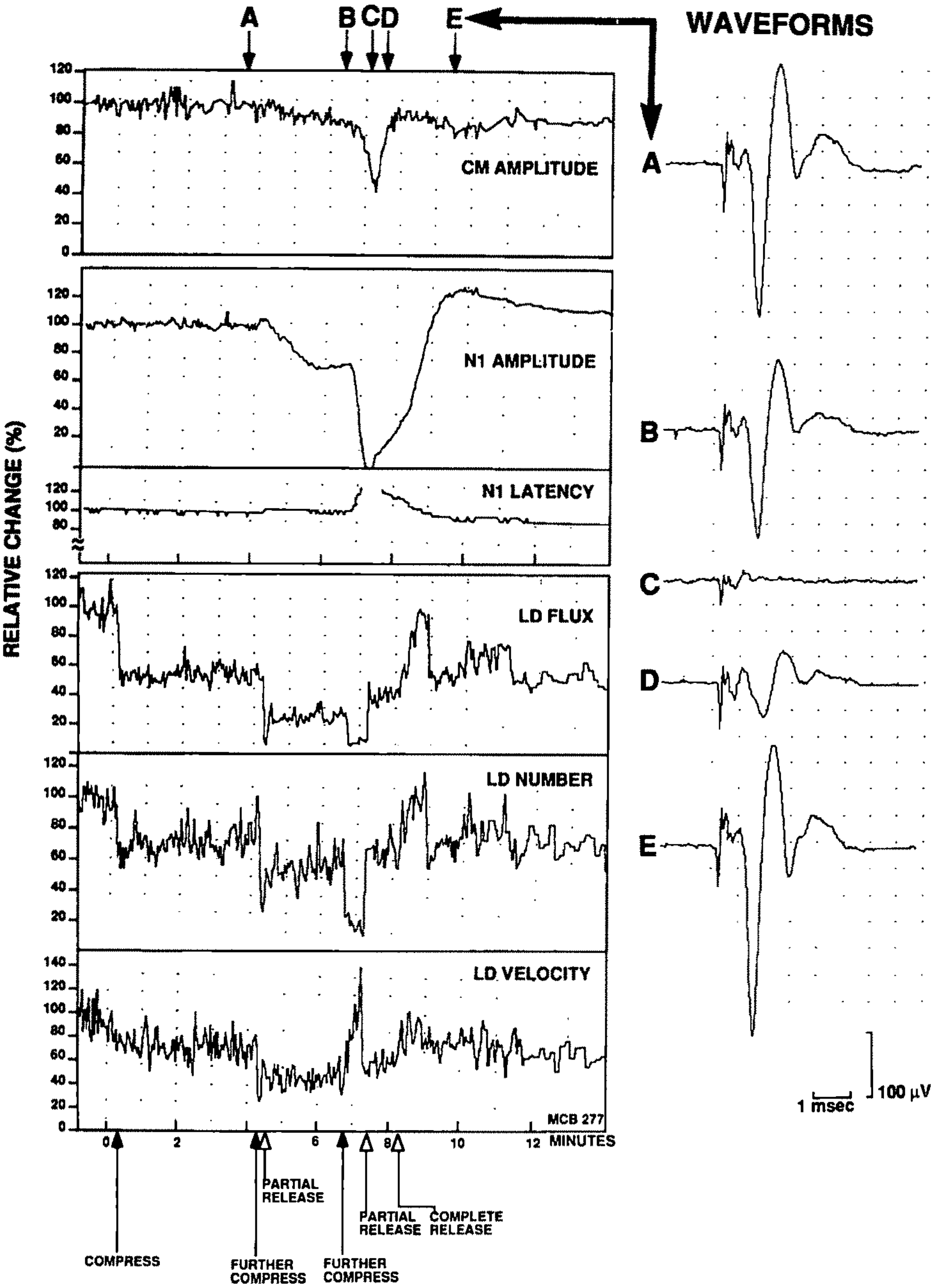

Fig 3. Effect of successive graded compressions and releases of eighth nerve complex on electrocochleographic potentials and laser-Doppler (LD) measurements. Compressions were achieved by pushing 125-μm micromanipulator-held wire to successively more ventral positions in eighth nerve complex. Amount of compression was adjusted to achieve stepwise changes in LD flux. Decrease and then increase in LD velocity seen with last compression were seen in other compressions in this particular guinea pig; however, increases in LD velocity with compression were not seen in other preparations. Drops in LD flux and LD number at 9 minutes are "spontaneous," in that they were not obviously caused by movements of wire used for compression. N1 latency was not plotted when N1 amplitude reached zero, because no N1 was recognized. Note that after complete release, N1 amplitude reached level higher than its level before any compression (see also text). This series of compressions was fourth compression in this preparation. Electrocochleographic waveforms on right (A-E) were obtained at times corresponding to arrows at top of Figure. CM — cochlear microphonic potential.

of these animals we cut the eighth nerve complex at the internal auditory meatus in an attempt to interrupt all cochlear blood flow through the internal auditory canal. In one guinea pig, the LD flux decreased by about 50% and remained at a lower level for about the next 11 minutes (Fig 1[26]). About 2 minutes after the cut, LD flux increased slightly and some of the high-frequency fluctuations of the LD flux that had been eliminated by the cut returned. (High-frequency fluctuations in LD flux have been correlated with the cardiac cycle.[26] Our measurements of the high-frequency fluctuations in LD flux, number, and velocity indicate that they are of the appropriate fundamental frequency — about 5 Hz — to be correlated with the cardiac cycle in guinea pigs.) In the other guinea pigs, the cut decreased LD flux by 75% and 80%. Blood pressure did not change (range 31 to 41 mm Hg). Only when the animals were painlessly killed (by clamping the trachea or injecting an intracardiac anesthetic) did the LD flux fall to zero and the high-frequency fluctuations disappear. As expected, N1 was completely and immediately lost with the cuts and never recovered. The CM amplitude rapidly decreased by 70%, and continued to slowly decrease, even into the period after death. Brief compressions of the eighth nerve complex (Figs 2-4) also resulted in complete loss of N1, but decreases in LD flux were never total. These results are consistent with the LD measurements at the cochlear capsule, having a large component due to blood flow to the cochlea from the

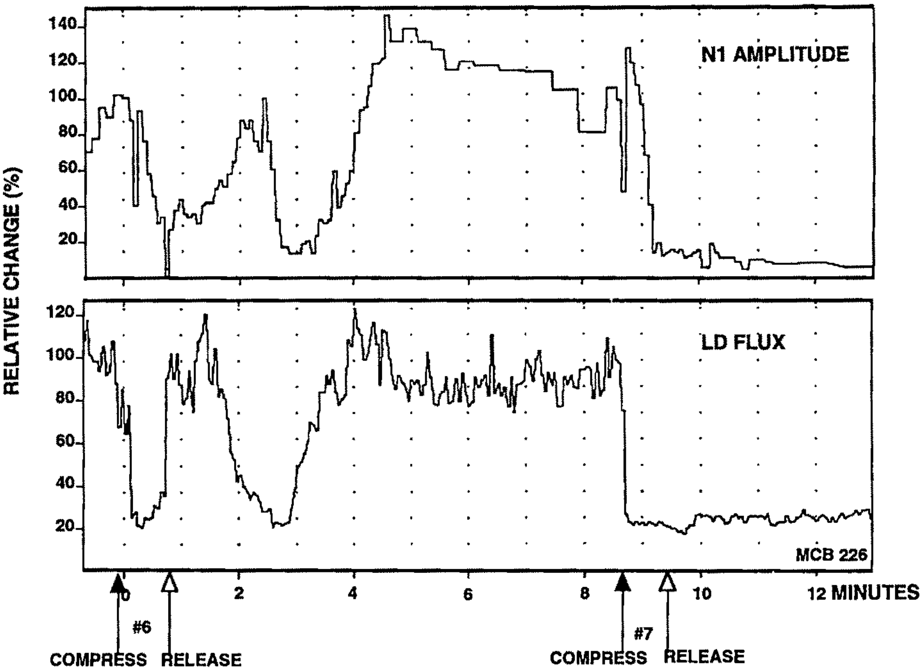

Fig 4. Instability of laser-Doppler (LD) flux and N1 amplitude during sixth and seventh compressions in guinea pig. Compression was applied to cochlear nucleus with blunt micromanipulator-held probe. Note that N1 amplitude and LD flux were fluctuating around 100% level prior to sixth compression. Release of sixth compression was followed by return of LD flux and N1 amplitude, but was immediately followed at 1.75 minutes by "spontaneous" decrease in LD flux that was in turn followed shortly thereafter by decline in N1 amplitude. Laser-Doppler flux then spontaneously recovered, followed by recovery of N1 to supernormal level. After seventh compression, release was not accompanied by return of LD flux or N1 amplitude.

internal auditory canal. However, it appears that there are contributions from other sources, which are not interrupted by compression of the eighth nerve complex, but disappear at death.

To observe the dynamics of the relationship between compression, LD measurements, and N1, in six guinea pigs the eighth nerve complex was compressed until the N1 was undetectable, and then released. A typical result is shown in Fig 2. With compression, the LD flux, number, and velocity abruptly decreased; with release they quickly returned to their precompression values. During compression, typically the high-frequency component of the LD measurements also decreased. The N1 decreased with compression, but always lagged behind the LD measurements; the first change in N1 began about 10 to 20 seconds after the change in LD measurements. With release of compression, the LD measurements immediately recovered, but the return of N1 was much slower, showing two distinct time courses. After release there was typically a very slow rise in N1 amplitude, followed by a much more rapid growth to complete recovery (Fig 2).

Results from two experiments showed that by varying the degree of compression, it was possible to progressively decrease the LD measurements and electrophysiologic responses (Fig 3). The first compression shown in Fig 3 caused decreases in LD flux, number, and velocity, while not affecting N1 or CM. A further compression (soon followed by a partial release, in order to obtain only a small decrease in LD flux) gave rise to greater decreases in the LD measurements that were accompanied somewhat later by slowly progressive decreases in N1 and CM that reached 30% and 15%, respectively. The waveforms of the electrophysiologic responses are shown on the right side of Fig 3. The final compression step resulted in greater sustained decrements in LD flux and number, but not in LD velocity. The LD velocity decreased at first, but *increased* within 10 seconds to a value above baseline. A similar pattern of decrease in LD flux, number, and velocity, with subsequent increase in LD velocity, occurred in this same preparation whenever a compression resulted in complete loss of N1, including when the eighth nerve complex was cut. Increases in LD velocity were seen in one other preparation, but were not typical of compressions in the four other preparations. The final compression step of Fig 3 was followed by a decline and then complete loss of N1 that recovered after release of compression. As N1 was declining in amplitude, its peak latency increased from 1.2 to 1.5 milliseconds. The CM declined in amplitude, but proportionately less than N1 and with a somewhat slower time course. After partial and then complete release, N1 amplitude recovered with at first a slow and then a more rapid time course (as was seen in Fig 2). After recovery, N1 amplitude was "supernormal" (ie, greater than its precompression value) for minutes in this and in other experiments. The second negative deflection in the compound action potential, N2, was also supernormal in amplitude. During this period, a DC offset occurs during the part of the waveform immediately preceding N1 (Fig 3, waveform E). This offset may represent an increased summating potential. Supernormal CM amplitude was observed in one preparation, where it was most pronounced for longer

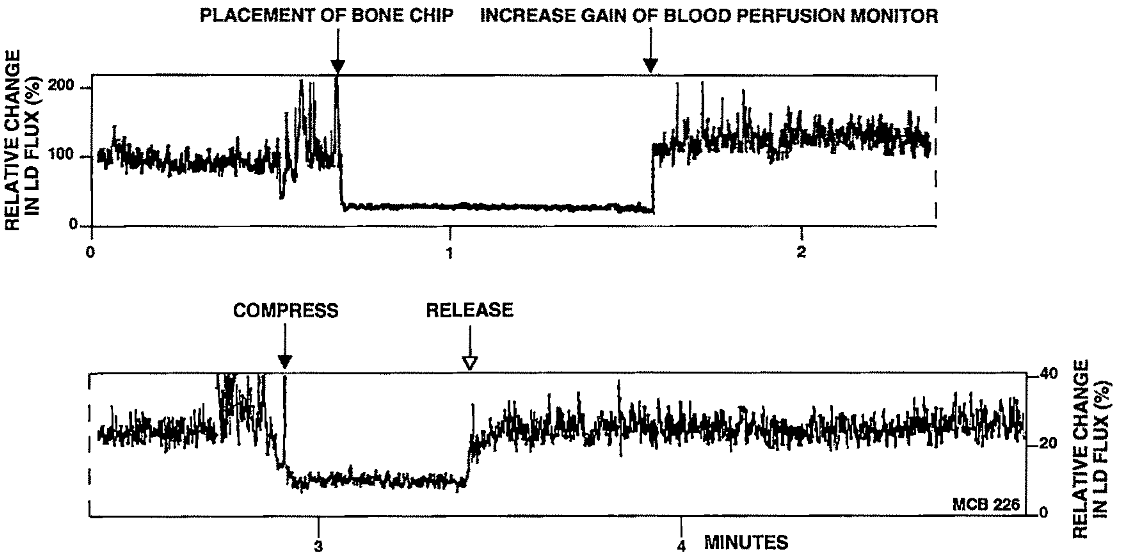

Fig 5. Strip-chart recording of laser-Doppler (LD) flux when 0.3-mm-thick bone fragment was placed between LD probe and cochlea. At time indicated, LD gain was increased by factor of 5. (Note that ordinate scale on right reflects this change.) Decrease in LD flux caused by compression could still be detected. Compression was applied to cochlear nucleus with blunt micromanipulator-held probe.

compressions (1 to 8 minutes). The LD measurements, however, did not show periods of supernormality that could clearly be distinguished from background fluctuations.

The time courses of changes in N1 and CM with compression differed. The first detectable decline in CM amplitude began a few seconds after N1 amplitude began to decrease. After sufficient compression, N1 amplitude fell rapidly to zero, whereas CM amplitude showed an initial decremental phase that was rapid (although not as rapid as N1, and it does not reach zero) and was followed by a prolonged and much slower phase of decrement. Long compressions or cuts to the eighth nerve complex were necessary to observe the prolonged phase of CM decline. Some CM continued to be present for many minutes after death. In contrast to N1's prolonged, two-stage time course of recovery (even with brief compressions; Figs 2 and 3), CM recovery was rapid once compression was released, even with compressions for as long as 8 minutes.

In four experiments, brief compressions were repeated at least seven times in the same preparation. In two guinea pigs complete recovery to precompression conditions occurred with each compression. In a third preparation, with the first five compressions recovery was complete, but with the sixth compression the LD measurements and N1 became unstable (Fig 4). Rather than returning to and remaining at its baseline, the LD measurements fluctuated, and as in the changes in LD measurements induced by direct compression, N1 amplitude changes lagged behind the LD measurement changes. In this same preparation, there was no recovery of the LD measurements or N1 after the seventh compression was released (Fig 4). In a fourth preparation, LD measurements were reversible, but N1 amplitude was irreversibly reduced by about 30% after the eighth compression. These irreversible changes do not appear to be due to systemic factors, for two reasons. First, we measured blood pressure in the fourth guinea pig and there was no change. Second, in a series of guinea pigs in which only electrophysiology was measured, in four animals, at a time when N1 had irreversibly declined, N1 from the contralateral ear was normal.

A potential problem in making LD measurements from human cochleas is the thickness of the otic capsule that separates the spiral ligament from the middle ear, where the probe is positioned. In humans the thinnest point is at the promontory over the basal turn. For five human temporal bones we found the average thickness at this point was 0.9 mm (range 0.8 to 1.0 mm). In contrast, the thickness of two guinea pig otic capsules averaged 0.2 mm at the position of our LD probe over the basal turn. In two guinea pigs, by placing a bone fragment (from the squamous portion of a human temporal bone) between the probe and the guinea pig otic capsule, we investigated whether it was still possible to detect changes in LD flux with a thicker otic capsule. With bone fragments of 0.3-mm thickness, the amplitude of the LD flux measurement was greatly reduced by placement of the fragment, but could be compensated for by increasing the gain of the BPM 403A (Fig 5). The signal to noise ratio was still sufficient to detect the decrease in the LD flux with eighth nerve complex compression. The decrease in the high-frequency

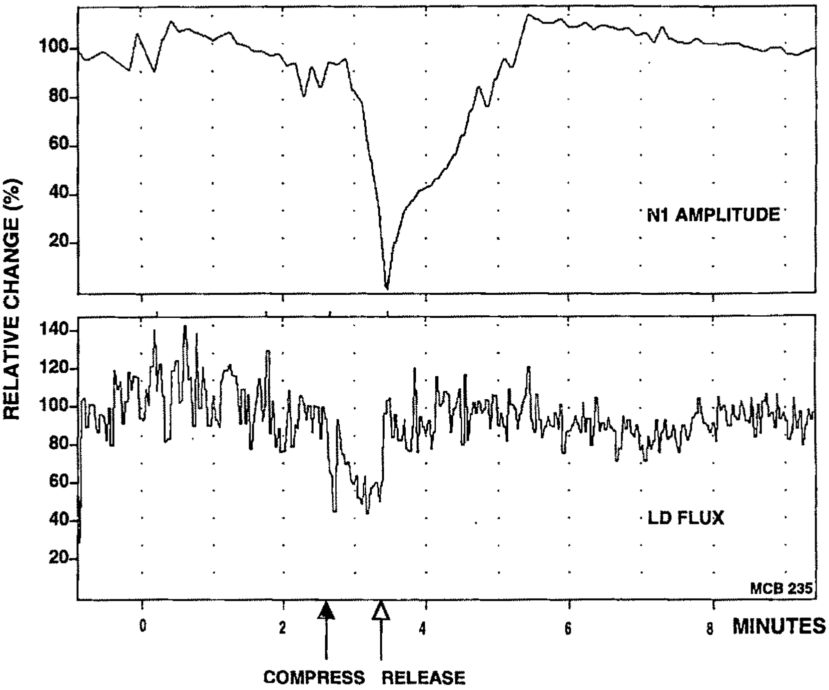

Fig 6. Effect of compression on N1 amplitude and laser-Doppler (LD) flux when 0.7-mm-thick bone fragment was placed between LD probe and cochlea. Together, thickness of bone fragment plus thickness of guinea pig otic capsule (about 0.2 mm) are equal to average thickness of human otic capsule near round window. Decrease in LD flux caused by compression could still be detected. Compression was applied to cochlear nucleus with blunt micromanipulator-held probe.

components of the LD flux during compression is well illustrated in Fig 5. Placing a bone fragment of 0.7-mm thickness between the probe and otic capsule appeared to cause a decrease in the signal to noise ratio, but the decrease in LD flux caused by compression was still measurable (Fig 6), since the LD flux clearly assumed a lower mean level during compression, while still fluctuating about this mean to about the same degree as during no compression.

DISCUSSION

Using a guinea pig model of human cerebellopontine angle surgery, we have shown that compression of the eighth nerve complex can lead to an immediate decrease in LD flux, number, and velocity, followed somewhat later by a loss of the N1 and CM potentials. These results suggest that LD measurements during human acoustic neuroma surgery, when combined with electrophysiologic recordings, may provide more benefit in the attempt to preserve hearing than the electrophysiologic recordings alone. Even though it is technically more difficult to make both measurements in humans, our preliminary results in humans are encouraging.

While there are many interspecies differences between humans and guinea pigs that might affect the recording of cochlear LD measurements from the middle ear, it is likely that the thicker bone of the human otic capsule will be an important factor. Scheibe et al[27] measured the attenuation of the laser beam by the human otic capsule and found it to be about four times that of the guinea pig otic capsule. Our results from interposing a 0.7-mm bone fragment between the probe and the otic capsule found a similar attenuation in the LD measurements (Fig 5). However, changes with compression could still be detected despite the attenuated signal. Scheibe et al[27] also reported human LD measurements from an unspecified location in the middle ear of four human subjects and found that the LD measurements varied with deep inspiration. Miller et al[28] noted LD measurement changes with electrical stimulation and caloric stimulation in humans, and argue that these LD measurement changes are from the cochlea. These results along with the present study suggest that LD measurements can, in fact, be used to detect human cochlear blood flow. The contention of Scheibe et al that the mucosa "may contribute up to 90 percent to the [human] cochlear laser signal"[27(p23)] suggests that in order to detect changes in human cochlear flow, the mucosa between the probe and the otic capsule may need to be removed.

The fact that LD measurements never fall to zero with eighth nerve compression in guinea pigs, even when the physiologic measurements were consistent with complete interruption of arterial flow, suggests that other motile sources were scattering the laser beam back to the detector and thereby contributing to the LD measurements. Whatever the source of these residual LD measurements, we have now shown that it is not from a source that can support cochlear electrophysiologic function.

Contributions to these residual LD measurements could come from blood flow through tissues within the field of the laser beam, such as the middle ear mucosa or bone of the otic capsule. However, there is evidence that mucosal flow in guinea pigs may not be a major component of the total flow. Others have reported that with the mucosa blotted away, compression of the eighth nerve[20] or obstruction of the basilar artery[29] can decrease LD flux by 70% to 90% of the baseline (roughly consistent with present results). However, since neither study simultaneously recorded any measure of cochlear function, such as ECochG, it is unclear to what extent these decreases in LD flux are due to alterations in cochlear blood flow or alterations in flow to other structures such as the otic capsule.

In our experiments the labyrinthine artery was not isolated and occluded, but instead pressure was applied to the eighth nerve complex, which includes as well afferent and efferent auditory nerve fibers, vestibular and facial nerve fibers, and sympathetic and possibly parasympathetic fibers. In view of the fact that the auditory nerve and vestibular nerve may be transected without affecting the CM or N1,[30] it is most unlikely that the effects on LD measurements and CM and N1 are due to direct compression of any of these nerves. Three facts support our contention that the effects we are reporting are principally due to compression of the labyrinthine artery and not other mechanisms, such as a change in systemic blood pressure. The first is that our electrophysiologic results are similar to those of Perlman et al,[8] who selectively compressed the internal auditory artery. The second is that we found no blood pressure changes at any time in the animal we monitored for blood pressure. Moreover, our LD measurements are similar to those of Short et al,[20] who also compressed the eighth nerve complex and noted no change in systemic blood pressure. The third is that with irreversible loss of N1 due to compression, we have always found that N1 was normal when recorded from the opposite ear.

Persistent, residual LD flux was recorded in our preparations, even when we severed the eighth nerve complex, thereby interrupting all flow in the labyrinthine arteries. In our compression experiments, however, the possibility of persistent residual arterial flow through incomplete compression of the labyrinthine arteries[12] exists, despite the fact that our compressions were sufficient to suppress the electrophysiologic responses as drastically as directly compressing the labyrinthine arteries[8] or asphyxiating the guinea pig.[31] Other possibilities for residual LD flow include another arterial source besides that through the internal auditory canal.[32] Movement of the preparation with respect to the probe, such as may occur with the animal's respirations and imperfect head fixation, could account for some residual LD measurements. Because the LD measurements went to zero with the animal's death, and because our click train has a small duty cycle, it is unlikely that acoustically induced movements of the membranous labyrinth could account for any residual LD measurements.[33] The most likely explanation may be persistent venous flow, since venous flow may not be interrupted by eighth nerve compression, because of the fact that the principal venous drainage of the guinea pig cochlea is through the vein of the cochlear aqueduct.[34]

Perlman et al[8] reported periods of supernormal flow after release of compression of the internal auditory artery in guinea pigs. Their methods consisted of direct observation of the blood flow through a fenestra in the apical otic capsule. Their Figure 3 is very similar to our observations of declining N1 and the CM with transection of the eighth nerve complex. They also observed that after release of arterial compression, N1 and the summating potential went through a "supernormal" period in which their amplitudes were greater than they were prior to compression. They attributed these changes to their observations of supernormal blood flow. We did not observe periods of supernormal LD measurements. The difference in the flow observations of these two studies may be explained by different 1) methods used for measuring flow, 2) durations of compression, or 3) locations of the measurements (basal versus apical turn). Supernormal periods of oxygen tension have been reported in one study of the cochlear fluids,[15] but not confirmed in other studies.[16]

Findings analogous to the increase in LD velocity with large compressions that was seen infrequently (in two of our preparations, eg, Fig 3) were not reported by Perlman et al.[8] In our measurements, LD flux was dominated by LD number rather than LD velocity. An increase in the velocity of the blood cells remaining after compression should be apparent with the method of direct visualization, unless there were very few cells with increased velocity, as suggested by our LD measurements. The possibility that this increase in LD velocity may represent a change in cochlear microcirculatory dynamics induced by complete cessation of arterial flow through the internal auditory canal deserves further study.

Our demonstration that cochlear electrophysiology parallels LD measurements at the guinea pig otic capsule provides further support for the contention that these LD measurements are indeed demonstrating blood flow from the cochlea. The fact that spontaneous fluctuations in LD measurements (such as shown in Fig 4) had the same effect on N1 as direct compression suggests that the LD measurement changes are not due to compression per se, but that 1) LD measurements are related to cochlear blood flow and 2) spontaneous fluctuations may be related to vasospasm or thrombosis involving blood vessels traumatized by the repeated compressions. In fact, the ECochG recordings made during compression of the eighth nerve complex in the cerebellopontine angle of the guinea pig are reminiscent of one pattern of ECochG recordings observed while monitoring acoustic neuroma surgery. Often, while the surgeon is working over the eighth nerve complex in the cerebellopontine angle, an abrupt decrease in N1 will occur, typically over about 2 minutes. In some cases the electrical potentials do not recover, but in other cases, recovery occurs when the surgical manipulations are halted. "Supernormal" amplitude can occur during this recovery (Figure 2 from Nadol et al[1] and Figure 6-10 from Levine[9]), as is typical in animal experiments after release of compression of the labyrinthine artery (Perlman et al[8] and present results). These similarities suggest that during acoustic neuroma surgery, LD measurements will show a decrease that precedes the ECochG changes. The time interval between 1) the LD measurement change due to complete occlusion of the internal auditory artery and 2) the electrophysiologic change may be 10 to 20 seconds (the guinea pig pattern) or longer (up to 50 seconds), as based on an observation during human surgery when an artery was first compressed and then cauterized.[9] The extra time provided by LD monitoring will allow a more prompt response to cochlear ischemia, which could lead to better hearing preservation in acoustic neuroma surgery. Another potential benefit from LD monitoring during acoustic neuroma surgery is detection of transient decrements in the cochlear blood supply that are not severe enough to cause electrophysiologic changes (Fig 3, first compression) but that may indicate that the cochlear blood supply is at imminent risk. By clearly establishing a vascular cause for the hearing loss, LD measurements might lead to adjunct treatments, either preoperative or intraoperative, to increase cochlear blood flow or minimize the damage from ischemia.

As has been shown in this paper, there are two reasons why monitoring acoustic neuroma surgery with LD measurements and ECochG may be an ideal way of providing evidence that the LD measurements are actually a reflection of blood flow in the cochlea, rather than due to an artifact or blood flow in the mucosa or the bone of the otic capsule. One reason is that presently LD measurements cannot provide an absolute measure of blood flow, but can measure changes in flow, such as are anticipated to occur in acoustic neuroma surgery. The second reason is that by combining ECochG with LD measurements, changes in LD measurements can be correlated with changes in cochlear function. Such correlations will indicate that some portion of the LD measurements is due to cochlear blood flow. Once it is established that LD measurements can detect human cochlear blood flow, then within its limits[28] this technique can be applied to a number of disorders of unknown cause, such as idiopathic sudden hearing loss and Meniere's syndrome, to elucidate the role of cochlear blood flow in their pathogenesis.

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OFF-VERTICAL AXIS ROTATIONAL RESPONSES IN PATIENTS WITH UNILATERAL PERIPHERAL VESTIBULAR LESIONS

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Off-vertical axis rotation (OVAR) stimulates the otolith organs in a manner that is suitable for assessment of the otolith-ocular reflex. To further assess the potential clinical usefulness of OVAR, the eye movement responses of seven patients with surgically confirmed unilateral peripheral vestibular lesions were compared with the eye movement responses of a group of age-matched, healthy, asymptomatic control subjects. Patients and controls were tested with constant velocity rotations that followed a brief period of angular acceleration (velocity trapezoid) using either earth-vertical axis (EVA) rotation or OVAR. Both EVA and OVAR sinusoidal velocity profiles were also performed. Results indicated that each patient had 1) an asymmetric OVAR response, ie, a bias component whose direction was opposite normal when rotating toward the lesioned ear, and 2) a normal modulation component. Population data suggested that patients had 1) a more rapid decay of response than normal subjects during OVAR velocity trapezoids, 2) an increased phase lead as compared to normal subjects during sinusoidal OVAR, and 3) like normal subjects, a less rapid decay of response during OVAR velocity trapezoids than during EVA rotational velocity trapezoids. Taken together, these findings suggest that patients with unilateral peripheral vestibular deficits have abnormal otolith-ocular and semicircular canal–ocular reflexes but that a single labyrinth appears to provide an otolithic signal sufficient for qualitatively normal semicircular canal–otolith interaction.

KEY WORDS — acoustic neuroma, otolith organs, vestibulo-ocular reflex.

INTRODUCTION

Off-vertical axis rotation (OVAR) is a vestibular stimulus that can be used to assess the vestibulo-ocular reflex (VOR), including the semicircular canal–ocular reflex, the otolith-ocular reflex, and semicircular canal–otolith interaction.[1-3] As part of an ongoing effort to better define the role of OVAR testing in a clinical vestibular laboratory setting, we evaluated a group of patients with surgically confirmed unilateral peripheral vestibular lesions, an extreme asymmetry of peripheral vestibular function. Our goal was to identify markers of asymmetric peripheral otolith function with the hope of subsequently using OVAR to assess patients diagnostically. Previous testing with earth-horizontal axis (EHA) rotation[4] and OVAR[5] have revealed consistent alterations in otolith-ocular reflex responses as a result of complete unilateral peripheral deficits. However, our prior experience suggested that EHA rotation was inappropriate for routine clinical use because of the technical difficulty associated with performing such tests.

The goals of this study were 1) to compare results of OVAR to the results of EHA rotational testing in patients with unilateral peripheral deficits, 2) to assess the otolith-ocular reflex and canal-otolith interaction in patients with unilateral peripheral vestibular deficits, and 3) to compare the results of OVAR testing in the early postoperative period (ie, 6 weeks) to those in the late postoperative period (ie, 6 months).

METHODS

Subjects. The subject population for this study consisted of seven patients, six of whom had undergone surgery for removal of an acoustic neuroma and one of whom had undergone labyrinthectomy for Meniere's disease. Table 1 provides information regarding age, gender, side of lesion, and test dates for each of the patients. Each of the patients was evaluated twice postoperatively. For comparison with the VOR responses of patients, seven age-matched asymptomatic healthy controls were evaluated. All of the subjects and patients used for this study participated on a voluntary basis after testing was fully explained to them by a qualified research assistant, and they signed an informed consent form approved by the Institutional Review Board of the Eye and Ear Institute of Pittsburgh. Exclusionary criteria for the control subjects consisted of eye movement or postural responses outside of normal limits for our laboratory on the following tests.

1. An ocular motor screening battery that consisted of a search for nystagmus both with and without fixation and with horizontal and vertical gaze deviation, and an assessment of saccades, pursuit, and optokinetic nystagmus.
2. A search for static positional nystagmus in the

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TABLE 1. PATIENTS WITH UNILATERAL PERIPHERAL VESTIBULAR LOSS

Patient	*Age at Surgery (y)*	*Sex*	*Surgical Procedure*	*No. of Weeks Between Surgery and Test*	
				First Test	*Second Test*
1	24	F	Left acoustic neuroma resection	6	32
2	35	F	Right acoustic neuroma resection	9	29
3	57	M	Left acoustic neuroma resection	5	26
4	59	M	Left acoustic neuroma resection	12	25
5	37	F	Right acoustic neuroma resection	9	22
6	59	M	Right acoustic neuroma resection	7	21
7	70	M	Right labyrinthectomy for Meniere's disease	6	24

supine, head right, head left, right lateral, and left lateral positions with eyes open in the dark.

3. Responses to alternate binaural bithermal caloric irrigation using a closed-loop irrigator with water at 30°C and 44°C. In our laboratory a normal response is considered to be an asymmetry (reduced vestibular response) of less than 25% and a directional preponderance of less than 30%.

4. Ability to remain standing under a variety of sensory challenges assessed with dynamic posturography using EquiTest (NeuroCom).

All patients and subjects underwent ocular motor, positional, earth-vertical axis (EVA), and OVAR testing. The EVA testing made use of a conventional rotatory chair mounted on a turntable with 28 foot-pounds of torque. Rotational velocity was driven by a PDP 11/73 computer. All testing was performed eyes open behind darkened goggles with the use of electro-oculography. Eye movement signals were amplified by a DC-coupled amplifier with a cutoff frequency of 40 Hz and recorded on chart paper by the 11/73 computer with a sampling rate of 100 Hz. A calibration was performed before each trial by instructing the subjects to look at targets placed ±10° horizontally.

Earth-Vertical Axis Rotation. Velocity trapezoids consisted of a slow angular acceleration of 1.5°/s/s for 60 seconds to a constant velocity of 90°/s that was maintained for 60 seconds, then a rapid deceleration of 100°/s/s to stop the rotation. Testing was performed both with and without postrotatory head tilt. For the postrotatory head tilt trials, the same rotational velocity trapezoids were used, but immediately on cessation of rotation, the subjects were pitched forward at the waist by a technician to an angle of 45°.

Sinusoidal stimulation employed single frequency sinusoids at 0.02, 0.05, 0.1, and 0.5 Hz at a peak velocity of 50°/s. For each frequency, subjects were positioned prior to rotation such that their mean orientation was nose-up. At least three cycles of rotation were obtained at the lowest two test frequencies, and at least six cycles of data were obtained at the other, higher frequencies.

Off-Vertical Axis Rotation. The OVAR testing was performed with a device designed and built at the Eye and Ear Institute of Pittsburgh.[3] Briefly, the OVAR device consists of a rotation chair affixed to an 80–foot-pound turntable attached to a tilt stand. Rotation rate was driven by a PDP 11/73 digital computer, while angle of tilt was controlled manually with a hydraulically powered linear actuator.

For this study, the subjects were tilted before the trapezoidal or sinusoidal rotation profiles were performed. With the axis of rotation tilted 30°, patients first were tested with sinusoids of 0.02, 0.05, 0.1, and 0.5 Hz with a peak velocity of 50°/s. At least three complete cycles of stimulation were delivered for the lowest two frequencies, and at least six cycles of data were collected for the higher frequencies. Following sinusoidal testing, patients underwent first a clockwise and then a counterclockwise trapezoidal rotation about a 30° tilted axis, starting in the nose-up position. These OVAR trials consisted of acceleration at approximately 100°/s/s until a constant velocity of 60°/s was reached. Subjects were then rotated for 90 seconds, thereby allowing the initial exponential decay of slow component velocity to occur and the modulation and bias components to be assessed. Subjects were monitored during OVAR testing, which was discontinued if they became too uncomfortable.

Analysis of Response. The horizontal eye move-

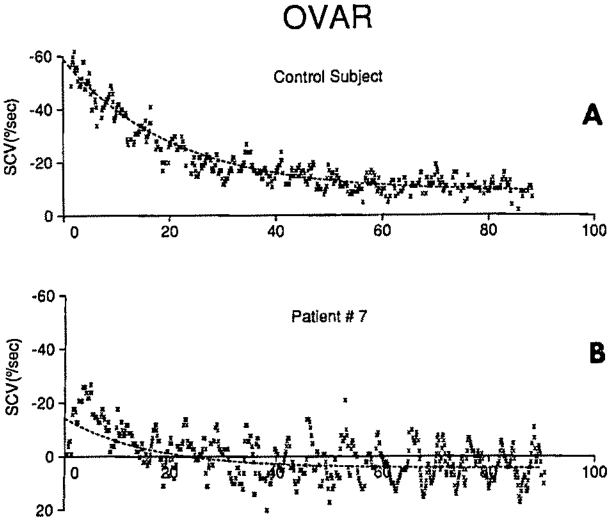

Fig 1. Slow component velocity (SCV) response to off-vertical axis rotational (OVAR) trapezoids. Dashed line indicates best fit of exponential decay plus bias of SCV response. A) Control subject. B) Patient 7, with right-sided unilateral peripheral vestibular deficit. Subjects were tilted to 30° nose-up position prior to onset of clockwise rotation. For SCV, negative (shown as up) corresponds to leftward slow components. OVAR responses have nonzero baseline (bias), which is negative for control subject and positive for patient. Vestibulo-ocular response time constant for control subject was 24.7 seconds, and for patient was 11.4 seconds.

ment response to both EVA rotation and OVAR was analyzed by first passing the eye position data through a nystagmus algorithm that identified the quick components of the nystagmus, interpolated across the quick component epochs, and then numerically differentiated the cumulative slow component eye position to estimate slow component eye velocity (SCV). For EVA rotational sinusoids, response gain and phase were estimated by using a least squares cycle-by-cycle fit procedure.[3] For EVA trapezoids, both with and without postrotatory head tilt, the dominant VOR time constant was estimated from the slope of the best straight line through a plot of the natural logarithm of the first 30 seconds of postrotatory slow component velocity versus time. For constant velocity OVAR trials, the per-rotatory slow component velocity was used to estimate the VOR time constant.

The OVAR bias component (ie, the nonzero baseline eye velocity) and the OVAR modulation component (ie, the periodic fluctuation of slow component eye velocity at the frequency of rotation) were estimated by using the slow component velocity elicited during the constant velocity period of trapezoidal rotation. Analysis was accomplished by least squares fitting the following equation through the data:

$$SCV(t) = amp\ e^{-t/\tau} + bias + modamp\ \sin(2\pi f + \phi)$$

where amp and τ are the amplitude and time constant of the initial exponential decay of the slow component velocity, bias is the magnitude of the bias component, modamp and ϕ are the magnitude and phase of the modulation component, and f is the frequency of rotation, which equals the reciprocal of the number of seconds per revolution.

For sinusoidal OVAR trials, the gain and phase of the presumed semicircular canal component of the response were estimated from the least squares fit of a sinusoid at the rotation frequency. With this type of OVAR stimulus, the (presumed) otolith components occur at higher frequencies; our analysis, by focusing on the semicircular canal component, addresses how the otolith signal during OVAR modifies the semicircular canal–ocular reflex.

RESULTS

Ocular motor testing was normal in each of the patients and subjects, except for a low-amplitude (less than 4°/s slow component velocity) spontaneous and/or positional nystagmus in three patients at the time of initial evaluation. The spontaneous nystagmus of one of these patients resolved; another patient, who had no discernible nystagmus initially, subsequently developed a low-amplitude nystagmus. The direction of the quick component of the spontaneous or positional nystagmus in each instance was away from the side of the lesion.

Six of the seven patients could complete constant velocity OVAR testing at the time of their early postoperative evaluation. All six exhibited a bias component that was always toward the lesioned ear, regardless of the direction of rotation. That is, for patients with right-sided lesions the bias component was positive (rightward slow component velocity; left-beating nystagmus) even when they were rotating toward the right. Figure 1B illustrates such an inappropriate bias component for a patient with a right-sided lesion. Shown for comparison is the OVAR response of a normal subject, for whom the direction of the bias component is opposite to the direction of rotation. At the time of their late postoperative evaluation, five of the seven patients could complete constant velocity OVAR testing. Four of the five had bias components that were in the same direction regardless of the direction of rotation; the direction was toward the lesioned ear for three patients and away from the lesioned ear for one patient.

The magnitude of the modulation component for each patient fell within 2 SD of the mean of the control population's modulation component. Figure 1B illustrates a representative example of a normal modulation component seen in the patient population (despite an abnormal bias component). The modula-

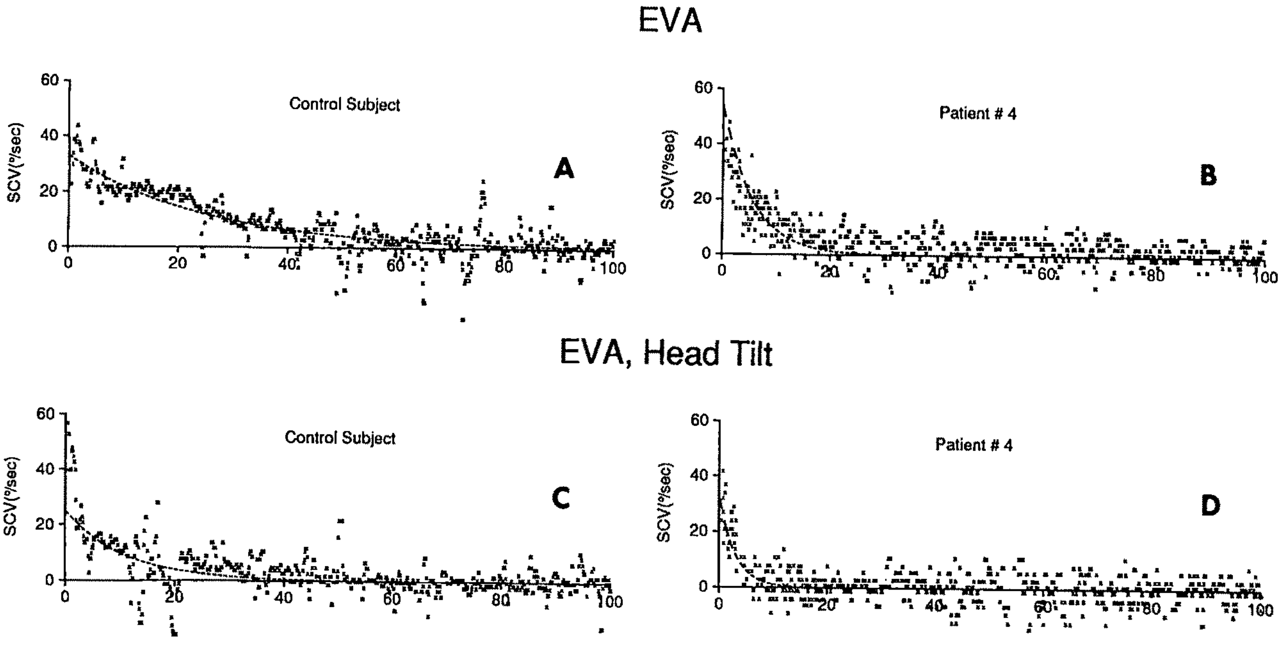

Fig 2. Postrotatory slow component velocity (SCV) response to earth-vertical axis rotational trapezoids with and without immediate postrotatory head tilt. All responses are following cessation of clockwise rotation at 90°/s/s. Dashed line indicates best fit of exponential decay of SCV response. A,B) Responses without head tilt. A) Control subject. Time constant was 27.3 seconds. B) Patient 4, with left-sided unilateral peripheral vestibular deficit. Time constant was 4.9 seconds. C,D) Responses with immediate postrotatory head tilt. C) Control subject. Time constant was 7.8 seconds. D) Patient 4. Time constant was 2.6 seconds. Note that patient's responses decay more quickly than those of control subject, and that responses with postrotatory head tilt have higher rate of decay than responses without postrotatory head tilt.

tion component magnitude for the patient population at the time of the initial evaluation (6.7°/s ± 1.1°/s) did not differ from that found at the second evaluation (6.1°/s ± 1.9°/s).

As expected, the rate of decay of postrotatory nystagmus following EVA trapezoids was higher for the patients than for the controls (Fig 2A,B). This same increased rate of decay of per-rotatory responses (ie, a shorter time constant) of the patients was also seen in the responses to OVAR trapezoids (Fig 1). The effect of postrotatory head tilt on EVA rotational trapezoids for a patient is shown in Fig 2D. The responses for a control subject are shown for comparison (Fig 2C). It can be seen in this example that the rate of decay of postrotatory nystagmus was shorter with head tilt as compared to without head tilt for both the patient and the control subject. This effect was seen for each of the control subjects and for five of the seven patients.

Population data for the first-order time constant of the VOR are shown in Table 2. Using two-way analysis of variance with repeated measures and a significance level of $p < .05$, we determined that overall, the time constant of the patients' responses was significantly different from that of the controls. Differences between patients and controls for EVA responses did not reach statistical significance; despite a large effect for EVA responses without head tilt, the sample size was too small for differences to reach statistical significance. However, differences between patients and controls for the time constant during constant velocity OVAR did reach statistical significance. A comparison of time constants at the initial evaluation with those at the second visit showed no significant differences.

We compared the responses of the patient population during sinusoidal rotations with those of the control population. The phase of the patients' responses was greater than that of the controls for both EVA and OVAR at all but the highest test frequency (Table 3). Gain was not consistently different between the patient population and the control population. For both gain and phase, we performed a statistical analysis of the data using a three-way analysis of variance (patient versus control, EVA versus OVAR, and frequency) with repeated measures and a significance level of $p < .05$. For phase, differences between patients and controls were statistically significant. We found a statistically significant difference between patients and controls for phase for both EVA and OVAR responses. A comparison of gain and phase at the time of the initial evaluation with those at the second visit showed no differences.

Although the primary aim of this study was to examine OVAR responses in patients with unilateral peripheral vestibular lesions, when we compared

TABLE 2. VESTIBULO-OCULAR REFLEX TIME CONSTANTS ESTIMATED BY USING ROTATIONAL TRAPEZOIDS

	EVA Rotation Without Head Tilt	*EVA Rotation With Head Tilt*	*OVAR*
Patients	7.4 ± 2.1 (n = 7)	5.7 ± 2.5 (n = 7)	12.6 ± 2.6 (n = 6)
Controls	14.0 ± 6.0 (n = 7)	5.1 ± 2.2 (n = 7)	23.6 ± 9.3 (n = 7)

Values, in seconds, are mean ± 1 SD. Note that EVA time constants were measured postrotatory, and OVAR time constants were measured per-rotatory.

EVA — earth-vertical axis, OVAR — off-vertical axis rotation.

OVAR with EVA responses we discovered two somewhat surprising results. First, the VOR time constant estimated by using constant velocity OVAR *exceeded* that during EVA, whereas the VOR time constant estimated by using the phase lead during low-frequency sinusoidal OVAR was *less* than or equal to the time constant estimated by using the phase lead during low-frequency sinusoidal EVA. With a two-way analysis of variance with a significance level of $p < .05$ and a Bonferroni correction for three comparisons (OVAR versus EVA without postrotatory head tilt versus EVA with postrotatory head tilt), the difference in time constants between OVAR and EVA without postrotatory head tilt was statistically significant. (Note that a 50% larger acceleratory stimulus was used for estimating the EVA time constant; this, however, is unlikely to explain the significantly larger OVAR result.) Although, in general, phase was increased during OVAR as compared to EVA rotation (Table 3), differences between OVAR and EVA phase were not statistically significant. Second, the gain during sinusoidal OVAR was decreased as compared to the gain during sinusoidal EVA rotation (Table 3). With a three-way analysis of variance and a significance level of $p < .05$, differences between OVAR and EVA gains were statistically significant. The difference in gain for OVAR versus EVA was statistically significant for both the patients and the control subjects.

DISCUSSION

This study using OVAR corroborates data obtained previously using EHA rotational testing that suggested an abnormal otolith-ocular reflex in patients with unilateral peripheral vestibular deficits.[4] Specifically, an asymmetric bias component was observed in both studies; with OVAR testing, the direction of the bias component was inappropriate in each patient (at the time of initial evaluation), whereas with EHA rotational testing, the bias component was either smaller (and of proper sign) when rotating toward the lesioned ear or was in an inappropriate direction. The modulation component during OVAR was found to be normal, in agreement with both EHA rotational[4] and linear acceleration[6] studies of patients with unilateral peripheral vestibular deficits. Also in agreement with EHA studies,[4] a shorter time constant was observed in patients with unilateral peripheral vestibular lesions as compared to controls. It is not surprising that responses to OVAR and EHA rotation are similar, since EHA rotation is actually a form of OVAR that uses a tilt angle of 90°, which generates a larger-amplitude stimulus than that generated by smaller off-vertical tilts, eg, the 30° tilt used in this study.

The asymmetric bias component in patients with unilateral peripheral vestibular lesions is consistent with previous data obtained with both OVAR[5] and EHA rotation.[4] The origin of the bias component during OVAR is unknown, but has been postulated to be a result of central nervous system processing of the population activity of otolith afferents.[7,8] The significance of the inappropriate bias component in patients with unilateral peripheral vestibular lesions is uncertain; the inappropriate sign of the bias component during OVAR when rotating toward the side of

TABLE 3. SINUSOIDAL ROTATIONAL RESPONSE PARAMETERS

		0.02 Hz		*0.05 Hz*		*0.1 Hz*		*0.5 Hz*	
		EVA	*OVAR*	*EVA*	*OVAR*	*EVA*	*OVAR*	*EVA*	*OVAR*
Gain (eye velocity re head velocity)	Patients	0.34 ±0.09*	0.18 ±0.03†	0.44 ±0.14	0.41 ±0.14	0.58 ±0.2	0.40 ±0.14	0.57 ±0.14	0.43 ±0.13
	Controls	0.4 ±0.14	0.24 ±0.10	0.52 ±0.14	0.31 ±0.16	0.51 ±.18	0.33 ±0.13	0.52 ±0.13	0.37 ±0.12
Phase (°)	Patients	42.3 ±7.7*	55.8 ±13.9†	21.0 ±4.2	26.8 ±7.4	10.3 ±5.0	18.5 ±9.1	−5.7 ±6.1	−6.5 ±14.2
	Controls	20.3 ±6.2	25.4 ±11.2	5.7 ±2.8	3.3 ±7.4	2.2 ±4.0	2.2 ±5.5	−4.6 ±4.5	−2.3 ±7.0

Data are mean ± SD; n = 7, except where indicated otherwise.

EVA — earth-vertical axis, OVAR — off-vertical axis rotation.

*n = 6.

†n = 3.

lesion may reflect an asymmetric dynamic otolith input to the central nervous system, and in this sense, like the directional preponderance of the bias component seen with EHA rotation,[4] could be regarded as a lateralizing otolithic sign. However, the inappropriate bias component might simply reflect an imbalance in central vestibular processing. The asymmetric bias component is unlikely to reflect simply a manifestation of spontaneous nystagmus, since only three patients had a low-amplitude spontaneous or positional nystagmus at the time of initial evaluation.

Our patients' responses to both EVA and OVAR were essentially unchanged at 6 months as compared to 6 weeks postoperatively. This suggests that the patients had compensated for their lesions as much at 6 weeks as at 6 months.

Although a larger number of patients will be required to establish the statistical significance of some of the effects seen in this study, our data suggest that a single labyrinth is sufficient to produce qualitatively normal semicircular canal–otolith interaction, as evidenced by the following: 1) during constant velocity OVAR, the per-rotatory VOR time constant was increased as compared to that observed during EVA rotational trapezoids in both patients and controls, 2) postrotatory head tilt decreased the VOR time constant in both patients and controls, and 3) for both patients and controls, the gain during sinusoidal OVAR was lower than the gain during sinusoidal EVA rotation. The longer per-rotatory VOR time constant seen during OVAR is thought to depend on otolith input to the velocity storage integrator,[2] a hypothetic neural circuit that causes vestibulo-ocular responses to persist beyond those of the vestibular periphery. The shortening of the VOR time constant with postrotatory head tilt appears to depend on static otolith input, which "dumps" activity in the above-mentioned velocity storage mechanism.[9,10] A comparable shortening of the VOR time constant with postrotatory head tilt has been seen in monkeys following unilateral otolithic lesions.[11] It is not clear why the sinusoidal gain should be decreased during OVAR, but we presume that this decrease is due to semicircular canal–otolith interaction.

The difference in VOR dynamics during OVAR versus EVA were in opposite directions for constant velocity as compared to sinusoidal stimuli. A possible explanation for this apparent inconsistency in our data relates to the effect of otolithic stimulation on the velocity storage mechanism. Previous studies have suggested that otolith input can either enhance ("charge") or diminish ("dump") velocity storage.[2] The lengthening of the VOR time constant during constant velocity OVAR may reflect enhanced "charging" of velocity storage. The shortened VOR time constant seen with postrotatory head tilt following EVA rotation may reflect velocity storage "dumping." These competing effects of otolith input on the velocity storage mechanism probably depend on the characteristics of the otolith input. Specifically, charging versus dumping of velocity storage may depend on "dynamic" versus "static" otolith inputs, respectively. "Dynamic" otolithic inputs can be produced by constant velocity OVAR or from other stimuli that produce a linear acceleration that changes direction continuously, such as counterrotation in a centrifuge.[12] "Static" otolith inputs result from head tilt when stationary. Our data suggest that 1) constant velocity OVAR increases velocity storage charging by combining a semicircular canal stimulus with a dynamic otolith stimulus and 2) that postrotatory head tilt causes velocity storage dumping by combining a static otolith input with a semicircular canal stimulus. However, our data also suggest that sinusoidal OVAR reduces the time constant of the velocity storage system (as evidenced by the increased phase lead of the VOR). That is, some types of otolith input that appear dynamic can actually diminish velocity storage rather than enhance it. Another example of a dynamic otolith stimulus that diminishes velocity storage is constant velocity EHA rotation.[13] Our findings suggest that the angle of tilt and the velocity profile of OVAR are important in determining the balance between velocity storage charging and dumping. Possibly, OVAR sinusoids of low enough frequency and amplitude will cause velocity storage charging, while constant velocity OVAR of high enough velocity will cause velocity storage dumping. Future studies will be required to pursue these ideas.

This study suggests that OVAR, a stimulus with an important otolith component, produces responses in patients with surgically confirmed total unilateral peripheral vestibular lesions that differ from the responses of normal subjects. The differences in the OVAR responses are those that would be expected from previous studies of the semicircular canal–ocular reflex and the otolith-ocular reflex of such patients. Specifically, the asymmetric bias component is comparable to the directional preponderance that has been reported from EVA[14-17] and EHA rotation[4] studies; the shortened time constant seen during constant velocity OVAR responses is comparable to the shortened time constant seen during EVA[14-17] and EHA rotation[4] studies; and the increased phase lead during sinusoidal OVAR is consistent with the increased phase lead reported with EVA rotations.[14-17] Taken together, the patients' responses suggest abnormalities of both the semicircular canal–ocular reflex and the otolith-ocular reflex. However, the preservation of the modulation component during constant velocity OVAR and the preservation of semicircular canal–otolith interaction suggest that our current OVAR test protocol is relatively insensitive to unilateral otolith dysfunction. Future studies using OVAR will be aimed at developing test protocols that are better able to detect such unilateral dysfunction.

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MASTERS IN OTOLARYNGOLOGY

The University of Pittsburgh otolaryngology faculty will be holding its Continuing Medical Education Program March 13-20, 1993, at the Sheraton Steamboat Resort in Steamboat Springs, Colorado. For additional information, contact Robin Wagner, The Eye and Ear Institute, 203 Lothrop St, Pittsburgh, PA 15213; phone 412-647-8783; fax 412-647-8720.

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EXPERIMENTAL TRANSFERABLE VASCULAR BED FOR LARYNGOTRACHEAL RECONSTRUCTION: FURTHER OBSERVATIONS

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Free fascia flaps have proven most reliable in providing a vascular bed for an epithelial free graft without adding bulk. They may be a useful tool for laryngotracheal reconstruction. A free flap consisting of a vascular network running in connective tissue can be developed on the rabbit external ear. The vascular characteristics of this flap were examined to test the reliability of the transferable vascular bed in laryngotracheal repair. The perichondrial free flap is useful for bringing an internal lining inside the lumen and for circumferential protection of supporting tissue. However, the natural tendency for surface contraction of perichondrium is a major disadvantage.

KEY WORDS — laryngotracheal reconstruction, vascularized perichondrium.

Free vascular fascia flaps have been used since 1985 for different types of reconstruction. Of these, the temporal, thoracodorsal, and radial forearm donor sites are the most popular. From the accumulated experience it became clear that these flaps have specific characteristics not available in other tissues.[1-4]

Free fascia flaps provide neovascularity without adding bulk and are particularly suited to serve in microvascular transfer of a recipient bed carrying an epithelial lining. These qualities may be useful in providing a mucosal lining at the interior side of the reconstructed larynx and trachea. A reliable technique for laryngotracheal reconstruction needs to fulfill the following requirements.

1. The reconstruction technique should be supported by a firm framework. The airway operates with both positive and negative pressure. An external skeleton is required to withstand the negative pressure of active inspiration.
2. It should have an epithelialized surface without subcutaneous tissue, in order not to narrow the airway lumen.
3. There should be a good blood supply to keep the reconstructed components viable.

The controversies and the different approaches in laryngotracheal reconstruction are due to the fact that these requirements are difficult to achieve. There are no donor sites available in the human body to provide an all-in-one technique for reconstruction of the larynx. Even free flap donor sites are restricted to anatomically privileged blocks of tissue that are reliably perfused by well-defined and surgically accessible large vascular pedicles.

A free fascia flap may be preformed in order to reconstruct a patch laryngeal defect or a circumferential tracheal defect by adding a mucosal graft for internal lining and also to bring in material for external support (Fig 1). The laryngeal patch could be

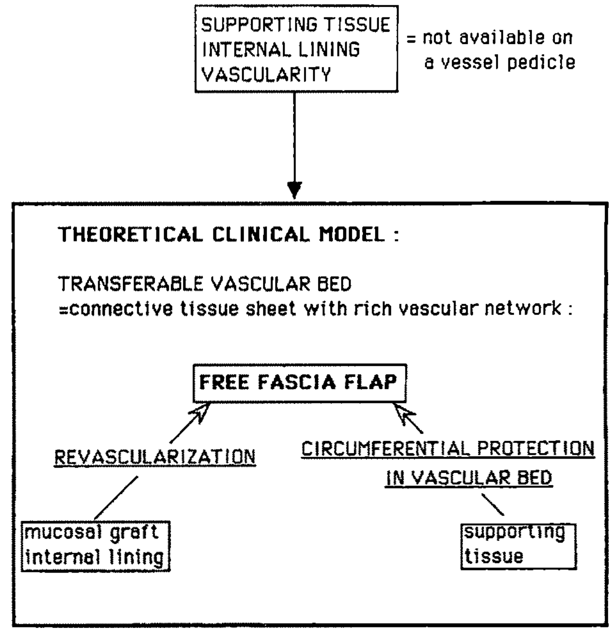
SUPPORTING TISSUE
INTERNAL LINING
VASCULARITY
= not available on a vessel pedicle
THEORETICAL CLINICAL MODEL :
TRANSFERABLE VASCULAR BED
=connective tissue sheet with rich vascular network :
FREE FASCIA FLAP
REVASCULARIZATION
CIRCUMFERENTIAL PROTECTION IN VASCULAR BED
mucosal graft
internal lining
supporting
tissue

Fig 1. Concept of theoretic laryngotracheal reconstruction with free fascial flaps.

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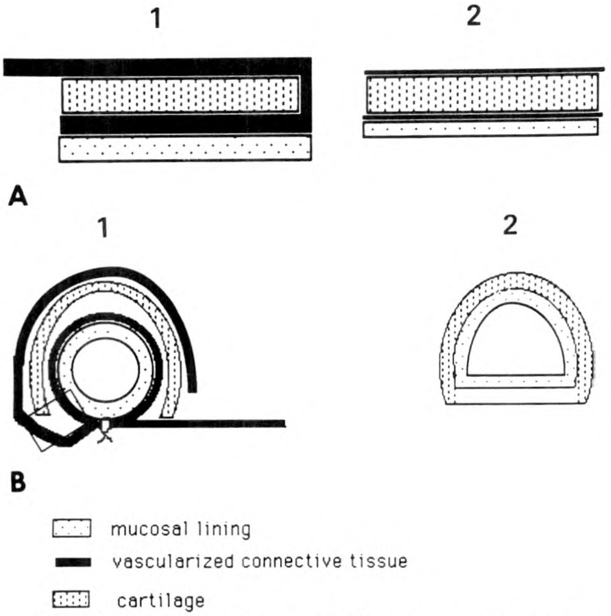

Fig 2. Laryngotracheal reconstruction. A) Laryngeal patch reconstruction with vascularized bed rotated 180° (1) next to normal laryngeal wall (2). B) Circumferential tracheal reconstruction with vascularized bed rotated 360° (1) next to normal tracheal tube (2). Histologic appearance of quadrangular detail can be seen in Fig 11C.

preformed by rotating the vascularized fascia 180°. In this way, the supporting material is circumferentially covered and protected within a vascular bed (Fig 2A). A tracheal tube may be created by rotating the fascia 360° in order to bring the mucosal graft internally and to enclose a tracheal cartilage allograft (Fig 2B). Essential for the success of the technique is a sufficient blood supply at the distal end of the flap to guarantee revascularization of the mucosal graft even after rotation of 180° or 360°. It was our purpose to look for an animal flap model with the same vascular pattern as the free human fascia flap. The rabbit auricular perichondrial flap contains a rich vascular pattern and is nourished from a central

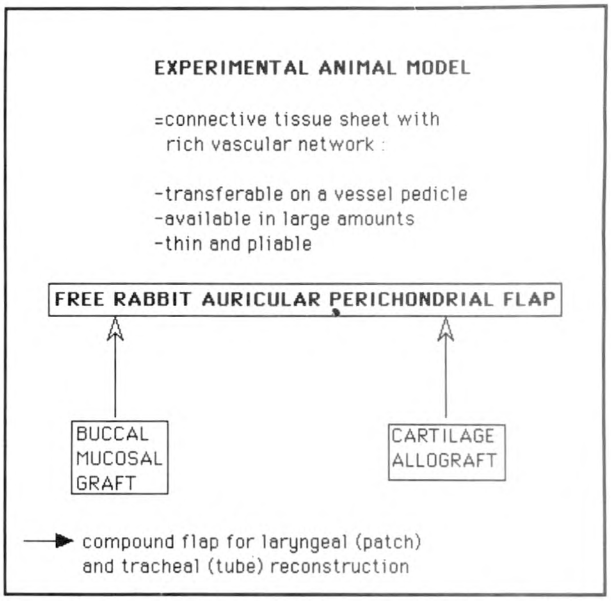
EXPERIMENTAL ANIMAL MODEL
=connective tissue sheet with rich vascular network :
-transferable on a vessel pedicle
-available in large amounts
-thin and pliable
FREE RABBIT AURICULAR PERICHONDRIAL FLAP
BUCCAL MUCOSAL GRAFT
CARTILAGE ALLOGRAFT
compound flap for laryngeal (patch) and tracheal (tube) reconstruction

Fig 3. Concept of experimental laryngotracheal reconstruction with free perichondrium flap.

vascular pedicle. This thin, pliable connective tissue sheet containing the vascularization can be transferred by microvascular techniques to the neck (Fig 3). The vascular characteristics of the flap were examined in detail in light of its previously reported usefulness for laryngeal reconstruction.[5]

MATERIAL AND METHODS

Flap Preparation. The animals were anesthetized with intravenous sodium pentobarbital plus inhalation of halothane, nitrous oxide, and oxygen. The dorsal auricular skin was removed, leaving the underlying vascular structures intact. Along the auricular skin, the soft tissues were incised, including the perichondrium. The perichondrial flap, measuring 5 x 12 cm, was detached from the cartilage by a periosteal elevator. The central auricular artery and vein

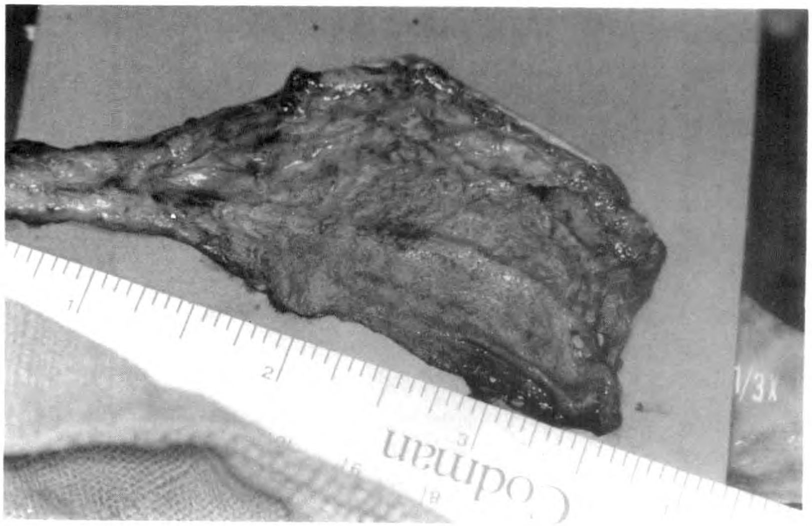

Fig 4. Dissected perichondrium flap on its vascular pedicle. Scale is in centimeters (length is 12 cm, width 5 cm).

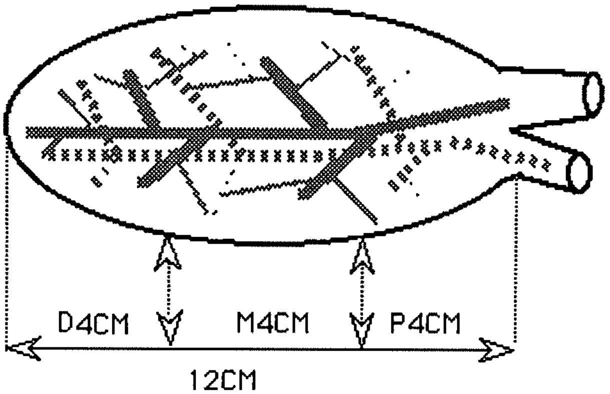

Fig 5. Perichondrium flap with three flap areas: proximal (P), middle (M), and distal (D) from vascular pedicle.

were dissected as a vascular pedicle for the flap (Fig 4). The arteries measured 0.9 to 1.2 mm, and the veins 1.0 to 1.8 mm.

We examined the distal blood supply of the flap and the influence of 180° to 360° rotation. Flap vascularization with a preserved vascular pedicle was examined in 10 animals. Measurements were done after dissection of the flap in the neutral position and after rotation of the distal end over 180° and 360° (over a tube) with the rotation point at 8 cm.

We examined flap vascularization with a preserved vascular pedicle and 360° flap rotation after a follow-up period of 4 weeks in five animals. A perichondrial flap measuring 12 x 5 cm was taken, with preservation of the vascular pedicle. The distal end was rotated over 360° (rotation point at 8 cm from vascular pedicle) over a silicone tube (diameter 0.7 cm). The ear donor site was closed over the flap. Measurements were done 4 weeks postoperatively after reopening the ear.

We examined flap vascularization after microvascular transfer and 360° rotation after a follow-up period of 4 weeks in five animals. The perichondrial flap was dissected and brought to the neck with reanastomosis of the central auricular vessels to the neck vessels (auricular artery end-to-side to common carotid artery, auricular vein end-to-end to external jugular vein; nylon 11.0). The distal end was rotated over 360° over a silicone tube. Measurements were done 4 weeks postoperatively after reopening the neck.

Laser-Doppler Flowmetry. Laser-Doppler flowmetry (LDF) provides a dynamic measure of blood flow as a voltage output that is proportional to the number and velocity of red blood cells in a measurement volume (normally 1 mm^3), ie, flux. It is based on the principle that monochromatic light scatters back from the microvascular bed, gaining a frequency (Doppler) shift that is proportional to the volume and velocities of circulating red blood cells. This principle is applied by directing a continuous laser beam to the tissue via an optic fiber, which then receives and carries the backscattered light to a photodetector. Emitted and backscattered light are continuously compared by a microprocessor to estimate blood flow in the tissue.[6] This weighted (normalized) light is analyzed and converted to a voltage. The penetration depth for the laser light has been estimated to be about 1 mm in human skin.[7]

A Periflux PF3 (Perimed, Stockholm, Sweden) was used for blood flow evaluation. Probe P436 was connected to an LDF measuring unit capable of generating a 5-mW 780-nm laser beam and analyzing the light scattering back from the tissue. To overcome movement artifacts, meticulous stability and complete hemostasis in the surgical area are essential. The flap was divided into three equal areas: proximal, middle, and distal areas of the vascular pedicle. The mean perfusion unit value for each area was calculated by averaging all the individual perfusion units that were collected during the first minute after stabilizing the probe. The measurements were made in the center of the ear by positioning the probe at the three discrete measurement sites. Relative values were calculated from the mean perfusion unit values. Body temperature was maintained at 37.5°C with a heating pad. Measurements were performed with a constant flap temperature of 35°C and controlled by the temperature setting of the PF3 laser-Doppler flowmeter.

Vascular Pattern. The vascular system of the perichondrial flap could further be visualized by washing out the flap with heparinized saline and perfusing it with india ink diluted 1:1 with 10% gelatin. The flap is then excised, pinned onto cardboard, fixed in 10% normal saline, cleared in cedar wood oil, and examined with a transmission light microscope.

Behavior of Free Mucosal Graft and Cartilage Allograft. Five laryngeal patches and five tracheal tubes were preformed at the donor ear site. The perichondrial flap was dissected on its vascular pedicle.

The laryngeal patch was created by turning the distal end over 180° with interposition of a Merthiolate-preserved thyroid cartilage allograft. The cartilage allograft was preserved for a period of 2 to 4 weeks. A full-thickness buccal mucosal graft measuring 1 to 1.25 cm was applied at the distal perichondrium with 9.0 nylon and fibrin glue. Two mucosal grafts were taken for the neotrachea formation and

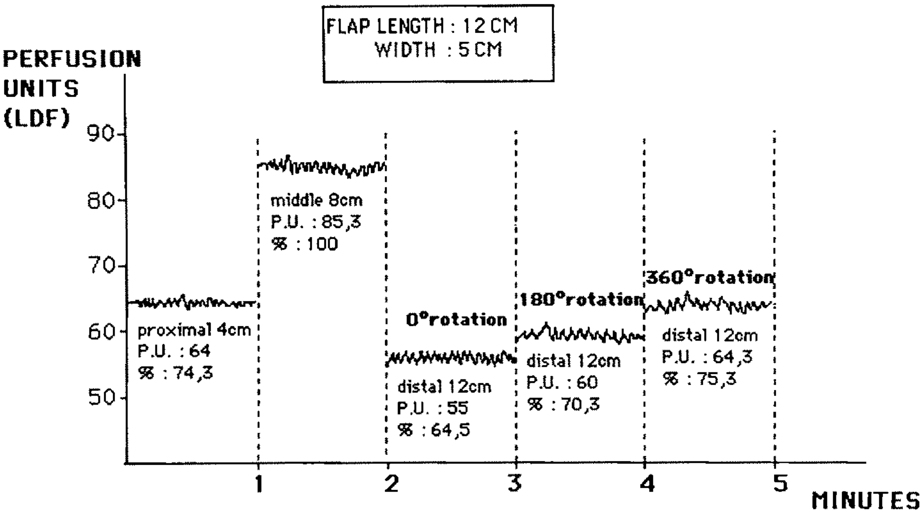

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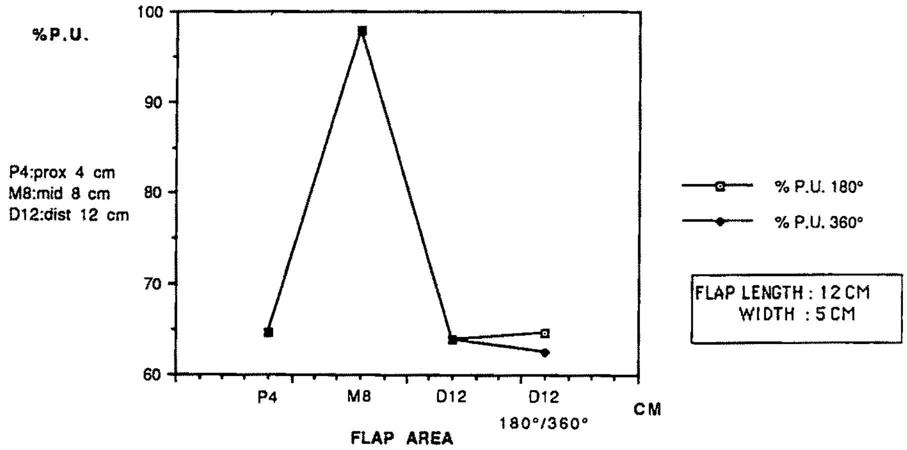
Fig 6. Laser-Doppler flowmetry measurements in group with preserved vascular pedicle before follow-up. A) Laser-Doppler flowmetry registration in rabbit 1. B) Mean relative perfusion unit (%P.U.) for each flap area with preserved vascular pedicle.



	P.U. 1	%	P.U. 2	%	P.U. 3	%	P.U. 4	%	P.U. 5	%
PROX	64	74,3	100	71,8	33,3	34,2	82,3	76,9	59	53,6
MIDDLE	85,3	100	139,3	100	97,3	100	107	100	85,7	77,9
DIST 0°	55	64,5	74	53,1	50,7	52,1	86,3	80,7	93,3	84,8
DIST 180°	60	70,3	80	57,4	61	62,7	78,7	73,6	110	100
DIST 360°	64,3	75,3	85	61	53,7	55,2	66	61,7	103,3	93,9

	6		7		8		9		10		MEAN	
PROX	49,3	36,4	187,3	85	91,3	63,8	65	77,3	83	72,6	**81,5**	**64,6**
MIDDLE	135,3	100	220,3	100	143	100	84	100	114,3	100	**121,2**	**97,8**
DIST 0°	113,3	83,5	129	58,6	81,6	57	45,7	54,4	56,7	49,6	**78,6**	**63,8**
DIST 180°	68,3	50,5	85,3	38,7	96,7	67,6	57	67,9	66,7	58,4	**76,4**	**64,7**
DIST 360°	95,7	70,7	68	30,9	89	62,2	54	64,2	57	49,9	**73,6**	**62,5**

B

applied one beside the other. The mucosal patch measuring 1.5 x 2 cm with its underlying perichondrium was circumferentially sutured around a silicone tube with a diameter of 0.8 cm. A tracheal cartilage allograft with a length of 1.5 cm was then brought over the reconstruction and sutured to the perichondrium with 8.0 nylon. The distal perichondrial remnant was sutured over the cartilage allograft. In this way a tracheal segment was preformed with a length of 1.5 cm. The external ear was closed around the preformed reconstruction. Treatment and care of the rabbits followed the rules of the American Physiological Society. Ambi-pen (penicillin G suspension, 30,000 U/100 mg) was administered for prophylaxis.

After a follow-up period of 6 weeks, the ear was reopened. The vascular pedicle was dissected free and the central auricular artery was injected with india ink diluted 1:1 with 10% gelatin. The rabbits were painlessly killed for histologic evaluation of the reconstruction site. All biopsy specimens were rou-

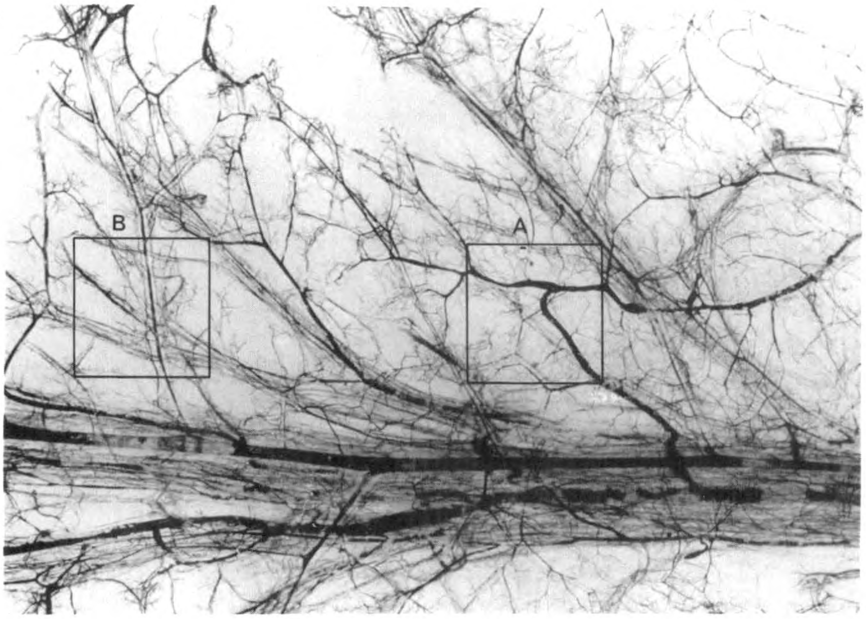


Fig 7. Vascular pattern of perichondrium flap with preserved vascular pedicle. A — detail of middle part, B — detail of distal part. Greater number and density of blood vessels in middle part.

tinely processed and stained with hematoxylin and eosin.

RESULTS

In the group studied for flap vascularization with a preserved vascular pedicle, measurements were done at 4, 8, and 12 cm (Fig 5). The LDF registration, mean perfusion units, and relative values within each group are presented in Fig 6. There were no statistically significant differences between the proximal and distal values at 0°, 180°, and 360° rotation. The middle part of the flap had the largest perfusion unit values ($p < .05$). This can be explained by the greater number of small blood vessels per surface area in the middle part of the flap, as can be seen after india ink–gelatin injection (Fig 7).

In the group with a preserved vascular pedicle and 360° rotation after a follow-up of 4 weeks (Fig 8), a constant observation was the tendency toward shrinkage of the dissected perichondrial flap. After 4 weeks, the mean flap length was reduced to 9 cm, and the width to 4 cm. The flap could easily be stripped from the cartilage after division of the adhesions between the cartilage and flap. Measurements were done at 3 cm (proximal), 6 cm (middle), and 9 cm (distal) in the center of the flap. No statistical differences were found among the perfusion values in the different

	P.U. **1**	%	P.U. **2**	%	P.U. **3**	%	P.U. **4**	%	P.U. **5**	%	**MEAN**	
PROXIMAL	68	100	51,3	88,4	22,3	53	44,3	66,1	54,7	100	**48,1**	**81,5**
MIDDLE	63	92,6	56	96,6	37	88	67	100	54	98,7	**55,4**	**95,2**
DISTAL	64	94,1	58	100	42	100	63,3	94,5	54	98,7	**56,3**	**97,5**
(360°)												

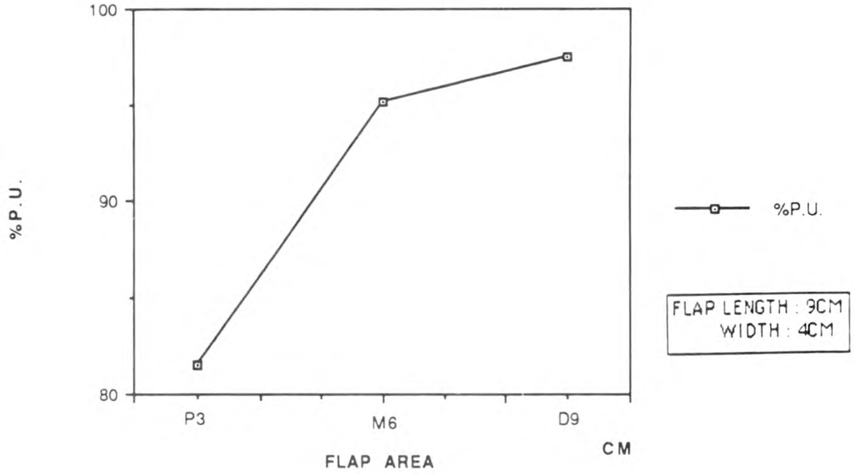


Fig 8. Mean relative perfusion units (%P.U.) for each flap area after follow-up (360° rotation). Group with preserved vascular pedicle.

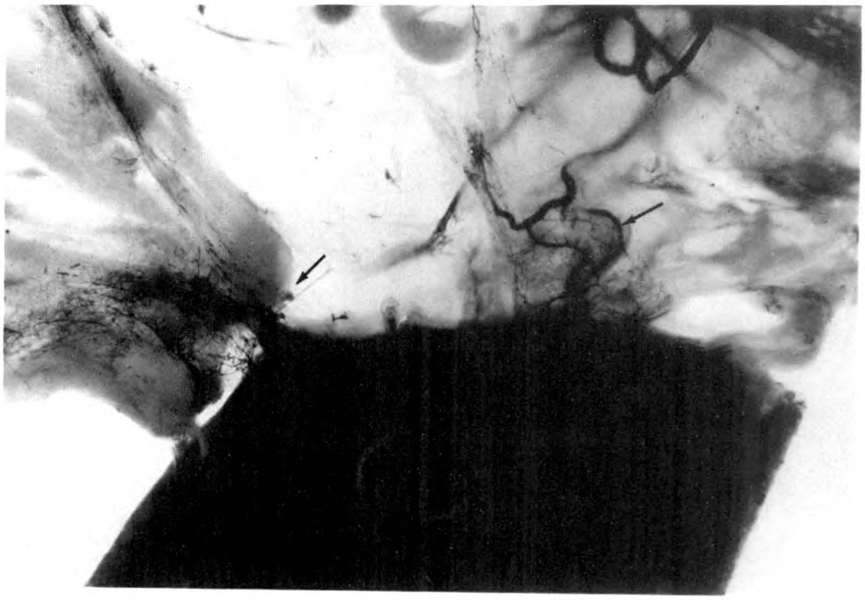


Fig 9. Vascular pattern of perichondrium flap after follow-up with outgrowth of capillary networks in connective tissue surrounding original flap (arrows).

flap areas. The vascular pattern displayed a capillary outgrowth within the flap and even in the neighborhood of the flap (neovascularity; Fig 9). There was no difference in vessel distribution among the different flap areas.

In the group examined for flap vascularization after microvascular transfer and 360° rotation after a follow-up of 4 weeks (Fig 10), a considerable shrinkage of the flaps took place, to approximately half the original size. The most involved flap segments were the proximal and middle parts, which were not fixed and were lying on top of the mobile neck muscles. Measurements were done at 2 cm (proximal), 4 cm (middle), and 6 cm (distal) in the center of the flap. No statistical differences in perfusion values were found.

The full-thickness mucosal graft was constant and completely viable in both the patch and tube reconstructions (Fig 11A). Less successful was the cartilage-preserved (Merthiolate 1:4,000 aqueous solution) allograft. In the majority of cases, areas of cell death were visible within the homograft, with early signs of resorption and loss of the support effect after a follow-up of 6 weeks. An inflammatory reaction was visible around the cartilage allograft. Patchy new cartilage formation was visible from the perichondrium in a cobblestonelike pattern. At other places, these patches coalesced and formed a more compact

	P.U. **1** %		P.U. **2** %		P.U. **3** %		P.U. **4** %		P.U. **5** %		**MEAN**	
PROXIMAL	41,3	63,5	76	84,4	51,7	100	64	100	57	100	**58**	**89,6**
MIDDLE	61	93,8	90	100	49	94,8	63	98,4	47,3	83	**62,1**	**94**
DISTAL (360°)	65	100	84	93,3	50	96,7	61,7	96,4	51,7	90,7	**62,5**	**95,4**

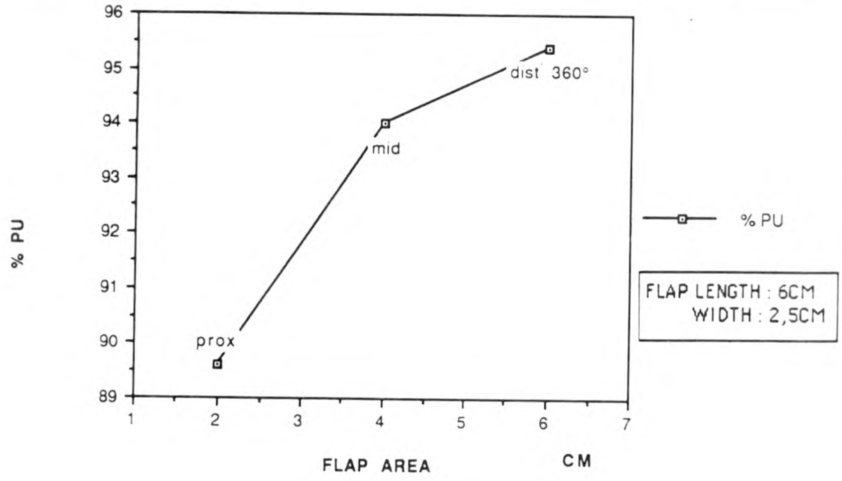


Fig 10. Mean relative perfusion units (% PU) for each flap area after follow-up (360° rotation). Group with microvascular transfer.

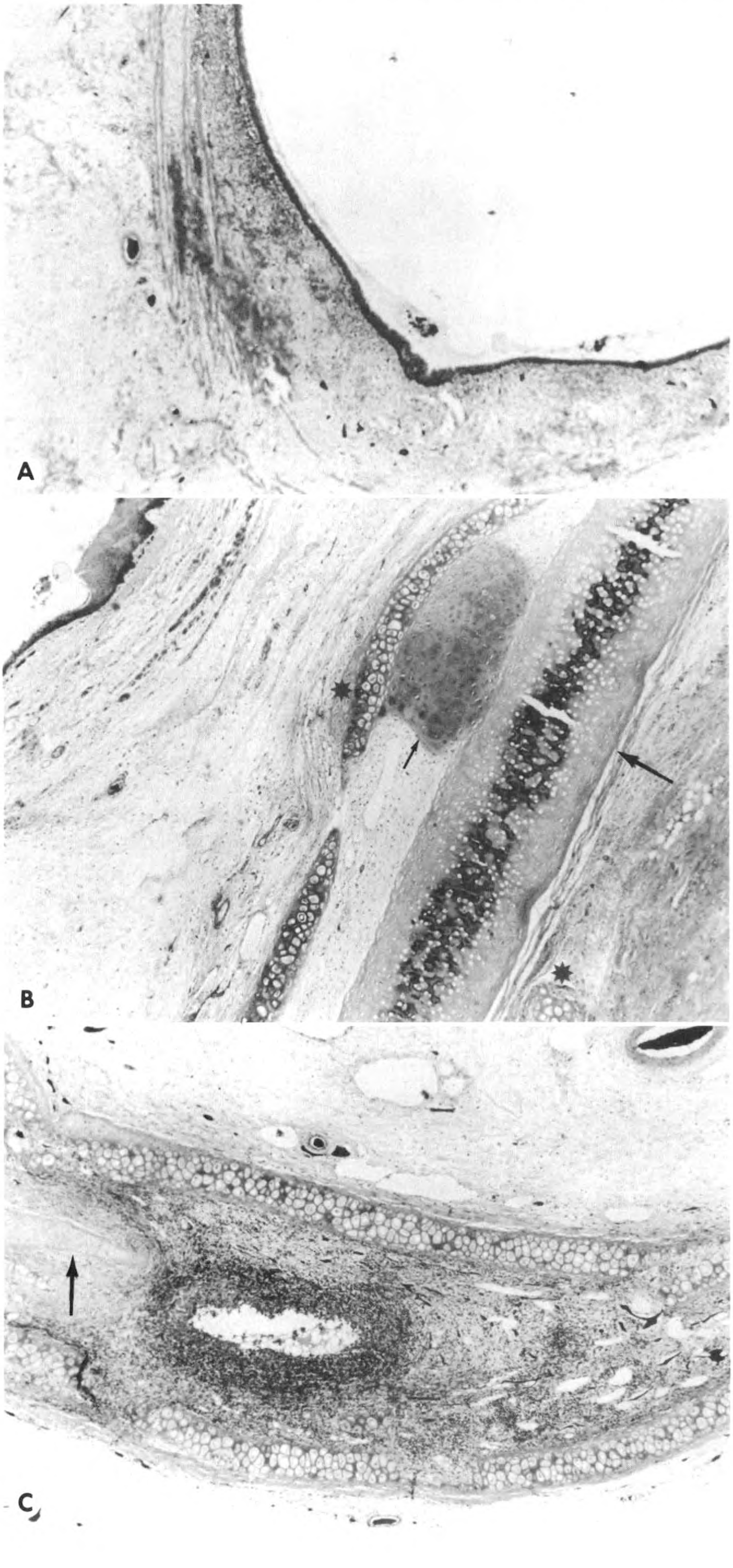


Fig 11. Photomicrographs of mucosal graft, cartilage allograft, and vascularized perichondrium. A) Oral mucosal lining in preformed tracheal lumen (H & E, original x25). Blood vessels were injected with india ink. B) Cartilaginous support in preformed airway wall after 6 weeks (H & E, original x20). Long arrow shows cartilage homograft with few chondrocytes within chondral lacunae and early signs of resorption centrally. In contrast, vital cartilage (short arrow) is small piece of ear cartilage taken at undersurface of perichondrium with dissection of flap. Asterisks — areas of new cartilage formation. C) Photomicrographic detail of Fig 2B after 6 weeks' follow-up (H & E, original x40). Double row of new cartilage formation surrounding end of cartilage homograft (arrow). Inflammatory reaction is visible around homograft with india ink–injected blood vessels, which are new vascular formations from original perichondrium.

plate (Fig 11B,C).

DISCUSSION

The thin, pliable nature and vascular characteristics of free vascularized connective tissue flaps seem most suitable for laryngotracheal reconstruction. Rotating the flap over 180° or 360° has no deleterious effect on distal vascularization. A well-vascularized surface is the best guarantee for take of the internal lining membrane. A full-thickness mucosal graft containing the entire thickness of the dermis is used for internal lining. To survive permanently, the graft has to become reattached and obtain a fresh blood supply from its new habitat. For take of the thick buccal graft, conditions have to be near-ideal: a rich blood supply of the graft bed, ensured by the distal perichondrium. Of importance, however, is the nature of the connective tissue holding the blood vessels together. Perichondrium has a natural tendency for shrinkage if separated from the underlying cartilage, to about two thirds or one half its original size. The underlying tissue plays a role in the degree of shrinkage, which is greater with an underlying mobile and elastic wall. The loss of flap surface area is less when new adhesions can be formed between the flap tissue and cartilage. This tendency of perichondrium flaps makes them unfit for use in repair of the laryngotracheal wall.

The long-term success of a cartilage graft depends on living chondrocytes that service and maintain the graft's bulk. Cartilage grafts preserved in Merthiolate undergo a progressive resorption with loss of external support. Early reports of cartilage homograft viability have to be taken cautiously.[5]

A new series of experiments is in progress, with preservation of the benefits (vascular characteristics of connective tissue flap, mucous graft) and improvement of the negative aspects (shrinkage of perichondrium, dead cartilage allograft). The lateral thoracic fascia is used as a transferable bed with autogenous cartilage for support.

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INTERNATIONAL HEARING AID CONFERENCE
GENERAL ANNOUNCEMENT AND CALL FOR PAPERS

An International Hearing Aid Conference will be held June 25-27, 1993, at The University of Iowa, Iowa City. For information on registration and accommodation, contact the Conference Center, University of Iowa, Memorial Union, Iowa City, IA 52242; (319) 335-3231, Fax (319) 335-3407. For information on Call for Papers, contact Regina Tisor (319) 356-2471, fax (319) 356-4547. We have applied for Continuing Education Units from ASHA and HAIC.

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HISTAMINE METABOLISM IN NASAL POLYPS

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We attempted to determine the relationship of nasal polyps to histamine (HA) metabolism. Compared to that in allergy-related nasal polyps and infection-related nasal polyps, the level of HA in aspirin-induced asthma–related polyps was significantly lower. Large differences between the groups were not observed in HA-synthesizing enzyme activity, but degradative enzyme activity was much higher in aspirin-induced asthma–related polyps than in other types of nasal polyps tested. These findings suggest the possibility that the amount of HA in polyps associated with aspirin-induced asthma was less because of greatly enhanced degradation. We found, in addition, that in nasal tissues such as polyps, histamine-*N*-methyl transferase, rather than histaminase, was the principal degradative enzyme.

KEY WORDS — aspirin-induced asthma, histamine metabolism, nasal polyps.

INTRODUCTION

The occurrence of nasal polyps as a clinical entity is quite familiar to otolaryngologists. Although they occur less frequently than in the past, nasal polyps are nevertheless often encountered in daily medical practice; their high rate of recurrence, in particular, is a cause for concern. In many cases, nasal polyps are complicated by sinusitis. The reasons for this association are not yet clear. Many factors are involved and various theories have been proposed,[1,2] among which the infection theory and the allergy theory are perhaps representative.

Histamine (HA) is present principally in mast cells and is distributed throughout the body. It has an extremely important role as a biologically active substance during infections and allergic reactions. Although there have been several reports[3,4] that HA plays a role in the onset of nasal polyps, none have related this role to HA metabolism.

In this study, we investigated the HA content in nasal polyps during aspirin-induced asthma (AIA) and in nasal polyps complicated by common sinusitis. We also studied HA-synthesizing and HA-degrading enzymes. We obtained several interesting results, reported here.

PATIENTS AND METHOD

Patients. Patients who underwent surgery for chronic sinusitis, nasal polyps, and hypertrophic rhinitis, who represented a subgroup of patients examined in the Department of Otolaryngology at Osaka University Medical School during the period October 1988 through August 1990, were used as subjects in this study.

Nearly all the operations were performed with local anesthetic (4% lidocaine hydrochloride, topically, with 1% procaine hydrochloride infiltration). However, 2% lidocaine without preservatives was used for anesthesia in cases of AIA. Excised tissues were immediately preserved by freezing to –80°C and analyzed later. The activity of HA and enzymes had already been confirmed to be stable at –80°C unless repeatedly frozen and thawed. In addition, the use of corticosteroid and nonsteroidal anti-inflammatory drugs was prohibited for all patients for the 3 days preceding surgery.

Diagnosis. As previously described,[5] diagnoses of AIA were made by using the patients' clinical history and the results of aspirin provocation tests. The presence of allergies was determined from the results of skin tests, radioallergosorbent tests, and tests for immunoglobulin E.

Histamine Metabolic Pathways. Histamine is synthesized from histidine by histidine decarboxylase (HDC). There are two catabolic pathways, one involving oxidative deamination by diamine oxidase (DAO; histaminase), and the other methylation by histamine-*N*-methyl transferase (HMT). Histamine is deactivated by these two enzymes.

Measurement Methods. Histamine was measured by using high-performance liquid chromatography (HPLC) and fluorometry with methods described by

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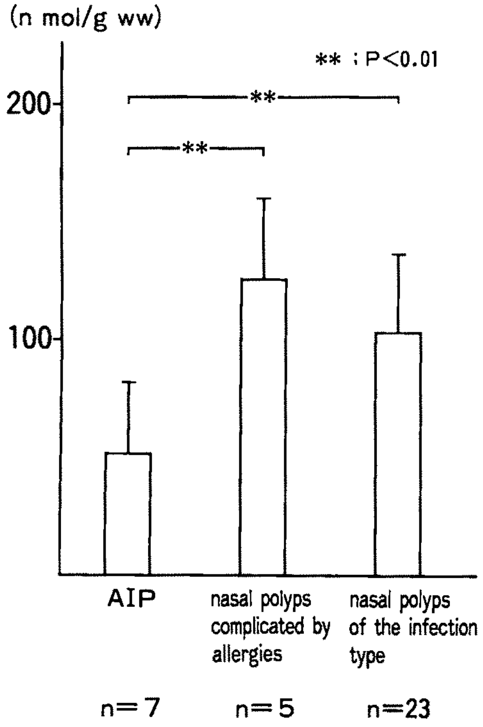

Fig 1. Histamine content in nasal polyps. Amount of histamine in cases of polyps associated with aspirin-induced asthma (AIP) was significantly lower than that of other two groups.

Yamada et al[6] and Yamatodani et al.[7] In brief, samples were homogenized in a phosphate buffer solution and centrifuged; perchloric acid was added to the supernatant; and measurements were made with HPLC after removal of the proteins.

The method for measurement of the activity of HA-synthesizing and -degrading enzymes has been reported.[8] In brief, for determination of HDC activity, the substrate histidine was added to a sample of the above-mentioned supernatant, and the HA produced by reaction at 37°C for 5 hours was assayed by HPLC and measured by comparison with a blank. As a blank, the specific HDC enzyme–inhibiting agent α-fluoromethyl histidine was added and the reaction product used.

For determination of HA-degrading enzyme activity, HA was added to a sample of the same supernatant and the amount of decrease was determined. That portion suppressed by the specific HMT-inhibiting agent SKF-91488 was regarded as HMT activity, and that part suppressed by the specific DAO-inhibiting agent aminoguanidine was regarded as DAO activity.

Statistical Analysis. The statistical significance of differences in means was determined with the nonpaired *t* test and regression analysis. Obtained p values less than .05 were considered to indicate significance, while p values less than .10 were taken as suggestive of significance.

RESULTS

Histamine Content in Nasal Polyps. There were 7 cases of nasal polyps associated with AIA (AIP), 5 cases of nasal polyps complicated by allergies, and 23 cases of infection-related nasal polyps (Fig 1). The HA content was 52.2 ± 29.5 (nmol/g wet weight) for the AIP cases, 126.3 ± 34.8 for the allergy-complicated cases, and 103.6 ± 34.5 for cases of infection-related nasal polyps. The mean amount of measured HA for the AIP cases was significantly lower than that for the other two groups. No correlation was found between the amount of sample and HA content.

Enzyme Activity in Various Tissues. Some of the cases in which enzyme activity was measured differed from those shown in Fig 1. The HA content and three types of enzyme activity were determined for 5 AIP cases, 2 cases of nasal polyps with allergies, 12 cases of nasal polyps not associated with infection-related allergies, 9 samples of maxillary sinus mucosa obtained from radical surgery of the maxillary sinus, and 4 samples of turbinate mucosa (see Table).

The mean HA content was again, among the nasal polyps groups tested, lowest in the AIP group, but no significant difference between group results was observed. The HA content was highest in the maxillary sinus mucosa and was significantly higher than in the AIP cases and the samples of turbinate mucosa. There was a tendency for the HA content in turbinate mucosa to be equal to or somewhat lower than that in nasal polyps.

ENZYME ACTIVITY IN VARIOUS TISSUES

	n	*HA Content (nmol/g wet weight)*	*HDC Activity (fmol/min per milligram protein)*	*(pmol/min per milligram protein)*		
				HMT Activity	*DAO Activity*	*HMT/DAO*
AIP	5	96.2 ± 27.0	20.0 ± 10.5	37.2 ± 23.7	0.221 ± 0.059	168
Nasal polyps CBA	2	103.9 ± 44.2	10.7 ± 5.2	9.9 ± 2.5	0.458 ± 0.470	22
IR nasal polyps	12	129.0 ± 83.7	25.0 ± 12.7	23.7 ± 20.7	0.312 ± 0.141	76
MS mucosa	9	197.0 ± 62.3	19.6 ± 12.2	17.3 ± 6.1	0.940 ± 0.564	18
Turbinate mucosa	4	86.0 ± 14.5	27.3 ± 9.1	26.7 ± 6.6	1.420 ± 0.597	19

HA — histamine, HDC — histidine decarboxylase, HMT — histamine-*N*-methyl transferase, DAO — diamine oxidase, AIP — polyps associated with aspirin-induced asthma, CBA — complicated by allergy, IR — infection-related, MS — maxillary sinus.

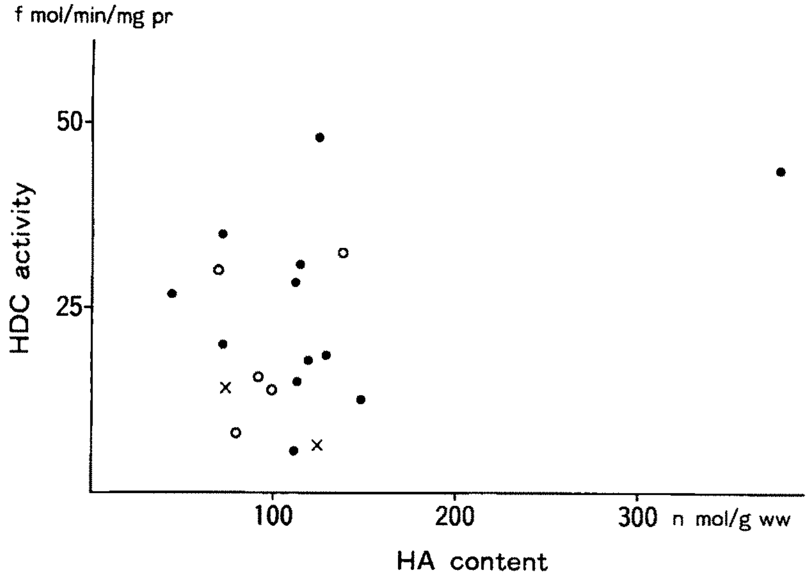

Fig 2. Correlation between histamine (HA) content and histidine decarboxylase (HDC) activity. No uniform correlation for group of nasal polyps was observed. Open circles — polyps associated with aspirin-induced asthma, crosses — nasal polyps complicated by allergies, closed circles — infection-related nasal polyps.

A uniform tendency among the five tissue type groups in the activity of the HA-synthesizing enzyme HDC was not observed. On the other hand, this activity was most enhanced in the turbinate mucosa, which had the lowest content of HA.

With regard to the activity of the degradative enzymes HMT and DAO, values for HMT were higher than those for DAO in each of the tissues examined. Although a uniform tendency among the various tissues tested was not observed for nasal polyps, particularly those in cases of AIP, the ratio of HMT to DAO was especially high. The HMT activity itself was especially high in cases of AIP.

Correlation Between HA Content and HDC Activity. A uniform correlation between HA content and activity of the synthesizing enzyme HDC was not observed when the group of all nasal polyps was tested (Fig 2).

Correlation Between HA Content and HMT Activity. A significant correlation was observed between HA content and HMT activity for nasal polyps not accompanied by allergies (Fig 3). Results suggestive of correlation between these two were obtained for cases of AIP as well. Since this tendency was marked for cases of AIP, and since HA degradation was pronounced within the nasal polyps tissues in cases of AIP, the low tissue HA values obtained may have been artifactual.

Correlation Between HDC Activity and HMT Activity. A significant correlation was observed between the activities of synthesizing enzyme HDC

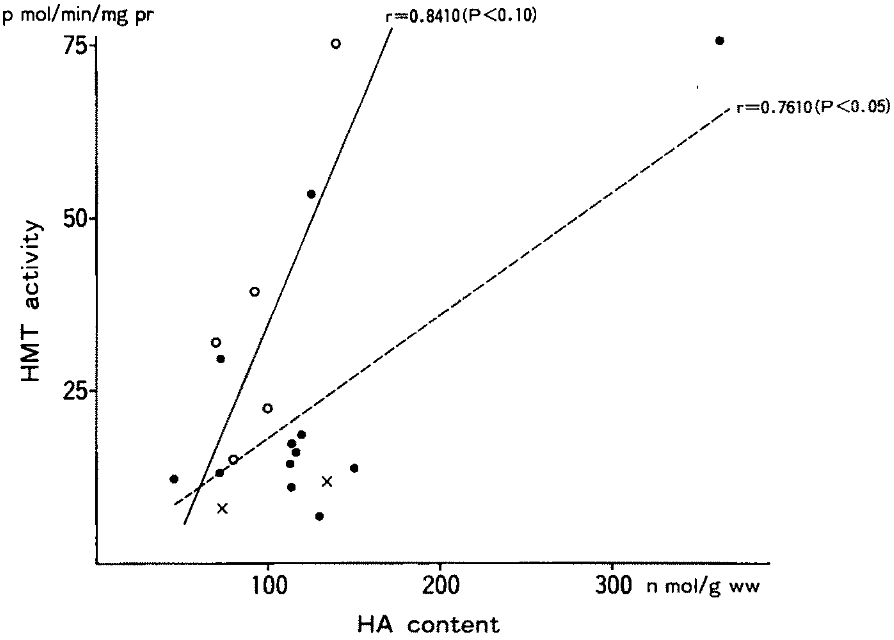

Fig 3. Correlation between histamine (HA) content and histamine-*N*-methyl transferase (HMT) activity. Significant correlation was observed for infection-related nasal polyps (r = .7610, p < .05). Tendency toward correlation was also observed for cases of aspirin-induced asthma (r = .8410, p < .10). Open circles, straight line — polyps associated with aspirin-induced asthma; crosses — nasal polyps complicated by allergies; closed circles, dashed line — infection-related nasal polyps.

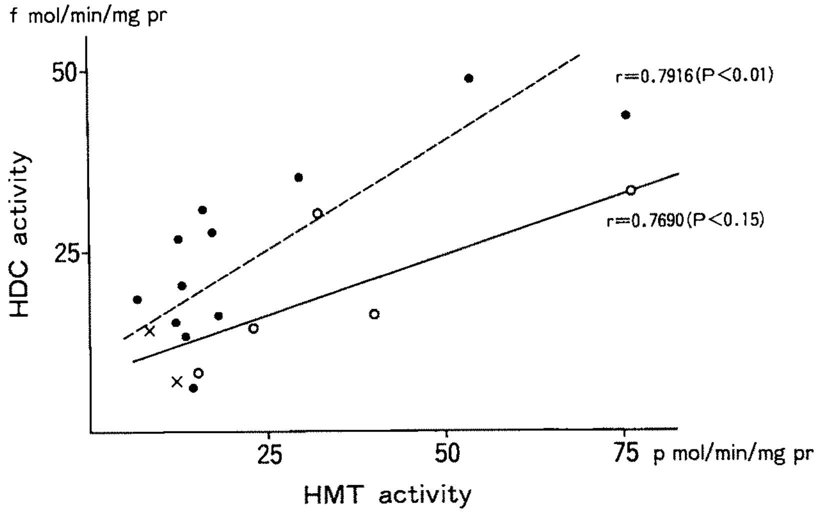

Fig 4. Correlation between histidine decarboxylase (HDC) activity and histamine-*N*-methyl transferase (HMT) activity. Significant correlation was observed in infection-related nasal polyps ($r = .7916, p < .01$). Open circles, straight line—aspirin-induced polyps associated with aspirin-induced asthma; crosses—nasal polyps complicated by allergies; closed circles, dashed line—infection-related nasal polyps.

and the degradative enzyme HMT in nasal polyps not accompanied by allergies (Fig 4). A tendency toward correlation was also observed for these two in cases of AIP was well. Using the line specified by the correlation, a tendency toward much greater degradative activity could be observed in AIA-associated polyps than in nasal polyps not accompanied by common allergies.

DISCUSSION

Histamine is a biologically active substance of considerable importance in the pathology of allergies and inflammations, and has been reported to be involved in the onset of nasal polyps.[3,4] In this study we measured the content of HA and of HA-synthesizing and -degrading enzymes in several tissues as a means of elucidating the causes of nasal polyps. There have been several reports[4,9,10] of the measurement of the HA content of nasal tissues, but few studies have been made simultaneously of HA-synthesizing and HA-degrading enzymes. Aspirin-induced asthma quite frequently complicates nasal polyps as a unique disease entity whose cause has not been elucidated.

Although HA is present in the nasal tissues, as has previously been reported,[3] its concentration in nasal polyps is higher than in turbinate mucosa. Although not determined in the present study, the HA content in the nasal mucosa of patients with nasal allergies is thought to be less than in nasal polyps.

It is of interest that the HA content is significantly less in AIP than in other nasal polyps. There is little involvement of HA in the onset of AIP, which is different from that of ordinary nasal polyps, but other biologically active substances are believed to be so involved. In addition, as will be discussed later, because the metabolism of HA is accelerated, the amount of HA is apparently less. Both inflammatory conditions and activity may have been responsible for the high concentration of HA in the maxillary sinus mucosa; reinvestigation of this possibility needs to be undertaken.

Some interesting results related to the metabolism of HA included the findings that the principal degradative enzyme in the nose was HMT rather than histaminase (DAO), and that the HMT-DAO ratio was much higher for cases of AIP. The clinical stage of disease was present during evaluation in most cases considered in the present study. Additional studies need to be carried out to determine what kind of relationship these findings have to pathologic changes. In relatively few reports[11,12] has the relationship of HA degradation and synthesis to histaminase been investigated; it should be viewed from the standpoint of pathways of HA degradation and synthesis in cases of nasal allergies.

The activity of the HMT-degrading enzyme for a given amount of HA in cases of AIP was clearly greater than that in ordinary nasal polyps. In other words, the degradation of HA was quite vigorous in patients with AIP, and conversely, it can also be said that the metabolism of HA was accelerated. These findings may be related to the low HA content mentioned previously. However, the number of AIP samples was too small to permit us to be certain of this conclusion. Clinically, AIP is quite responsive to treatment with steroids,[5] although a definite conclusion cannot be reached regarding the relationship of this clinical fact to the enhancement of HA metabolism noted in this study. Knowledge of these relationships may prove useful for the development of more effective drugs.

Notably, relationships of the results obtained to histopathology were not studied. Although no correlation between HA content, enzyme activities, and sample amounts was found, it seems likely that even in nasal polyps the majority of HA is contained in mast cells; the same is true for HDC. Because they were treated with Poritoron homogenizer (Kinematic, Lucerne, Switzerland), these cells were nearly all killed. Even if the mast cells did not contain the same amount of HA, it is necessary to determine the relationship of HA and HDC activity to histopathologic patterns in a tissue, such as mast cells. On the other hand, regions containing HDC have not been fully tested yet; we plan to do so using autoradiography.

The possibility that HA is a cause of nasal polyps must continue to be tested in a variety of fashions, including determination of the type of cells that have been infiltrated, the condition of activation, the content of the other biologically active substances, and the stages of the clinical course. In addition, when the role of chemical mediators such as these in pathologic processes is examined, it should be not only with regard to the amount present in tissues, but also with regard to metabolism and their synthesis and degradation.

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The postgraduate Pediatric Airway Endoscopy Course will be held May 5-8, 1993, at St Louis Children's Hospital, St Louis, Missouri. For further information, contact Marilyn Crawshaw at 314-454-2138. The course will also be held November 3-6, 1993, at The Children's Memorial Hospital, Chicago, Illinois. For further information, contact Geri Miseska at 312-880-4457.

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IMAGING CASE STUDY OF THE MONTH

ULTRASOUND DETECTION OF ANAEROBIC NECK INFECTION

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BEER-SHEVA, ISRAEL

Anaerobic infections of the neck are life-threatening conditions. Early diagnosis and prompt treatment are mandatory in order to prevent severe consequences.[1,2] The anaerobic nature of the infection is indicated by culture results, although at times culture confirmation may prove difficult.[1] Gas production is an important feature of anaerobic infections.[1] While this manifests itself with subcutaneous crepitus and emphysema in some cases, in others a deeply seated infection precludes the clinical detection of this sign. The demonstration of gas bubbles in soft tissues of the neck by a simple imaging technique is quite important in the early recognition of the anaerobic nature of the infection.

CASE REPORT

A 12-year-old boy presented with a severe left cervical pain and torticollis. A week prior to admission, he had suffered from tonsillitis and had been treated with oral penicillin.

On admission, he had mild fever (37.8°C). The left tonsil was congested. Induration along the left sternocleidomastoid muscle and torticollis were found. The rest of the findings on physical examination were normal. A routine blood count revealed leukocytosis of 10,000 with a shift to the left. A regimen of intravenous penicillin G (crystalline) 10,000,000 U daily was initiated. By the third day of hospitalization, swelling of the left side of the neck occurred and fluctuation was detected. In ultrasound examination of the neck, a deep neck abscess and thrombosis of the internal jugular vein were demonstrated. The latter diagnosis was made from a lack of response to the Valsalva maneuver, incompressibil-

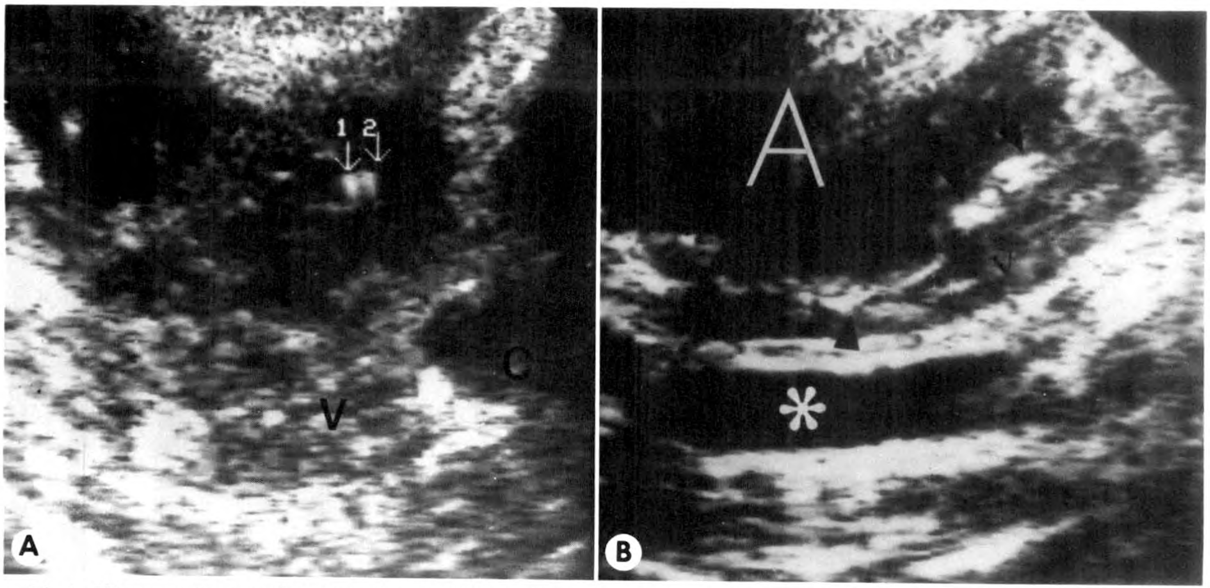

Fig 1. Ultrasound scans of left lateral neck. A) Transverse scan shows nonhomogeneous, ill-defined inflammatory mass. Carotid artery is patent (C) and internal jugular vein is obliterated (V). Strong flakelike echoes with comet-tail artifact (arrows) represent air bubbles. B) Longitudinal scan shows large nonhomogeneous abscess (A) with thrombosed internal jugular vein (V). Common carotid artery is seen behind vein (asterisk). Strong echoes without shadowing within abscess and adjacent to vein (arrowheads) are compatible with air bubbles.

From the Department of Otolaryngology (Tovi), the Ultrasound Unit (Barki), and the Radiology Institute (Hertzanu), Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

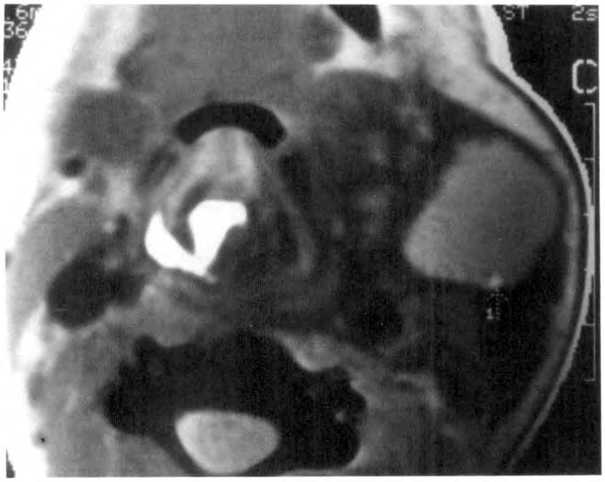

Fig 2. Axial computed tomogram (special window) at level of hyoid, demonstrating nonhomogeneous mass and neck abscess. Air bubbles are seen at periphery of abscess (arrow).

ity of the vessel, and echogenicity within the vein lumen. Additionally, small hyperechogenic foci adjacent to the vein wall (Fig 1A) and within the abscess (Fig 1B) were suggestive of gas bubbles. Computed tomography (CT) confirmed this diagnosis (Fig 2). The patient underwent drainage of the abscess and excision of the jugular vein. Postoperatively, intravenous metronidazole 1,000 mg was added to the initial antibiotic therapy according to the culture results of anaerobic streptococci. Two weeks later the patient was free of disease.

DISCUSSION

Anaerobic organisms are prevalent among the bacterial populations of the upper respiratory tract.[1] When they penetrate the mucosal plane they change their saprophytic behavior and become pathogenic. The early stages of anaerobic infections clinically manifest as banal infections.[2] Soon afterward they exhibit a fulminant course with extensive morbidity.[3] Deep neck abscesses, necrotizing soft tissue infections, mediastinitis, and thrombophlebitis of the internal jugular vein occur as consequences of anaerobic infections.[1] Anaerobic cultures are the mainstay in the recognition of these infections, but there may not be available appropriate specimens for anaerobic processing, and technical resources may be deficient. Furthermore, anaerobic bacteria are usually slow-growing, and culture requires a relatively long period of time.[1] Hence, appropriate treatment may be delayed.

Ultrasound delineates the main structures of the neck and detects various space-occupying lesions in this region.[4] Deep neck abscesses appear as complex masses along the fascial spaces.[5] Their echogenicity changes according to the amount of necrotic debris. In the present patient, besides the neck abscess, thrombosis of the internal jugular vein[6] was also ultrasonically demonstrated. Highly reflective, tiny echoes, without acoustic shadowing within the abscess and adjacent to the internal jugular vein, were suggestive of gas bubbles, as were confirmed by CT.

In ultrasound, large gas collections generate ill-defined acoustic shadowing and create difficulties in examination.[7] Small gas pockets, like gas bubbles, present as small, dense echoes,[7-10] usually without acoustic shadowing, because of a partial volume effect resulting from their small size.[7] This ultrasonic finding is not necessarily evidence of gas formation. Calcifications also manifest the same ultrasonic configuration.[7] Differential diagnosis between the two is made by radiographs or CT. In the present case radiographic studies of the cervical soft tissues did not show calcification. Furthermore, the presence of gas was confirmed by CT.

In conclusion, ultrasonic detection of small, dense echoes in patients with deep neck infection is suggestive of gas bubbles. This in turn raises suspicion of an anaerobic infection, prompt diagnosis of which is lifesaving.

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PATHOLOGY CONSULTATION

ADENOMATOID AND CALCIFYING EPITHELIAL ODONTOGENIC TUMORS

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The histologic diversity of the odontogenic epithelium's neoplasms is nicely illustrated by the adenomatoid and the calcifying epithelial odontogenic tumors. The former has a predilection for the maxilla, the latter for the mandible. Neither has a malignant variant, and each is controlled by conservative local removal. The recurrence rate of the adenomatoid odontogenic tumor is 0.2%, while the calcifying epithelial odontogenic tumor's rate is 14%.

The low incidence, preponderantly intragnathic location, and especially the multifaceted neoplastic potential of odontogenic epithelium place the surgical pathology of odontogenic tumors in the always-difficult category. The clinicopathologic features of two of these odontogenic tumors, the adenomatoid odontogenic tumor and the calcifying epithelial odontogenic tumor, are presented.

ADENOMATOID ODONTOGENIC TUMOR

The adenomatoid odontogenic tumor arises from odontogenic epithelium and is distinct from, and not just a histologic variant of ameloblastoma.[1] In terms of frequency among other odontogenic tumors, the adenomatoid odontogenic tumor is exceeded only by odontoma, cementoma, myxoma, and ameloblastoma.[2,3]

The tumor most often is seen in the anterior maxilla of girls (younger than 21).[1] It usually contains impacted teeth and calcific bodies, thereby presenting a rather distinctive radiographic appearance. Based on clinical and radiologic findings, there are three recognized variants: two central or intraosseous forms and a peripheral or extraosseous form. One central form is associated with the crown of an embedded tooth and is called the follicular type; the other central form lacks the crown association and is called extrafollicular.

In an international survey of 499 adenomatoid odontogenic tumors, Philipsen et al[2] found that 97.2% were central, and of these, 73% were follicular. Both central variants arose in the maxilla twice as often as in the mandible. Just over 50% of all variants occurred in patients who were in their teens (13 to 19 years). The extraosseous tumors present as swellings of the gingiva and are close or anterior to the permanent canines.[2] They show a 10 to 1 maxilla to mandible preference.[2]

All three of the variants show a close similarity with regard to age at discovery (mandibular tumors have a greater tendency to be found in patients over 30 years), gender preference (ratio of approximately 2:1 in favor of females), location, histopathology, and clinical course.[2] They also are all well controlled by a conservative surgical excision. Philipsen et al[2] could find only 1 tumor that recurred (the case was added in galley proof of their paper), thereby indicating a recurrence rate of 0.2% (1 in 500). This singular exception recurred several times and extended into the intracranial space.

Consistency of histologic appearance (Fig 1) characterizes the adenomatoid odontogenic tumor.[2,3] The most prominent feature is a dispersion of various-size solid nodules of cuboidal or spindle-shape epithelial cells in a loose stroma. The cells often resemble ameloblasts and form rosettes and whorls. Between the epithelial cells and in the center of the rosettelike aggregates there are eosinophilic droplets. Other findings are calcification (dystrophic, globular, cementumlike, concentric); an amyloidlike substance in the tubular spaces, about ducts, or intercellular; and micronodules of squamous-appearing cells. The cells are devoid of atypia and manifest only rare mitoses. Elsewhere in the tumor the cells can form a trabecular or cribriform pattern. Usually present are scattered ductlike spaces with a columnar cell lining. The nuclei in these cells are polarized away from the lumen.

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REPRINTS — John G. Batsakis, MD, Dept of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

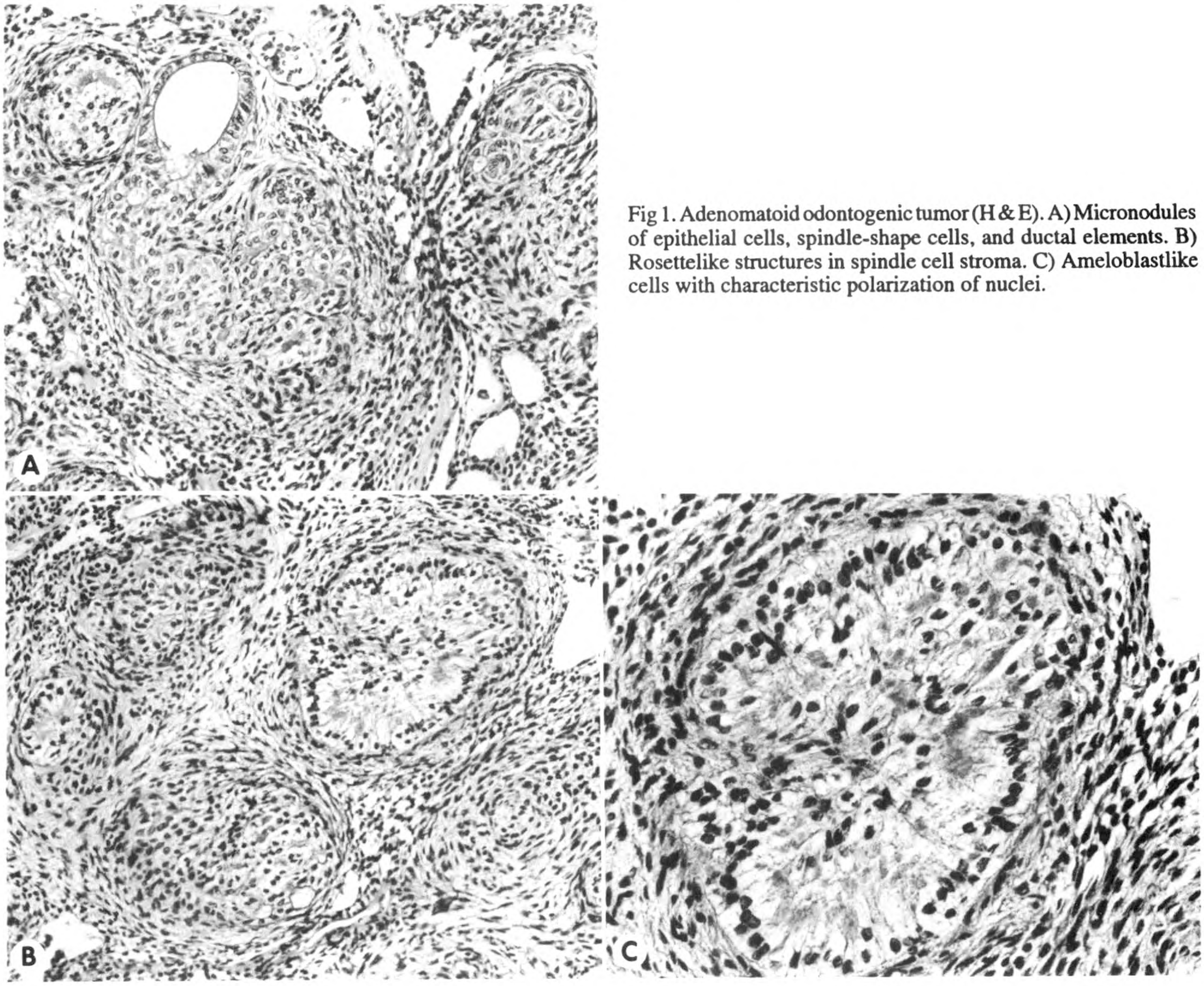

Fig 1. Adenomatoid odontogenic tumor (H & E). A) Micronodules of epithelial cells, spindle-shape cells, and ductal elements. B) Rosettelike structures in spindle cell stroma. C) Ameloblastlike cells with characteristic polarization of nuclei.

CALCIFYING EPITHELIAL ODONTOGENIC TUMOR

The calcifying epithelial odontogenic tumor, known also as the Pindborg tumor, accounts for about 1% of all odontogenic tumors.[4,5] Occurring over a wide age range (mean of 40 years) without gender preference, the tumor is typically a painless, slowly enlarging, intraosseous lesion with a predilection for the premolar and molar regions (molar prevalence is three times premolar).[5] There is a mandible-maxilla ratio of approximately 3 to 1.[4,5] An unerupted or impacted tooth (or teeth) is associated with the tumor in about one half of the reported examples.[5] Less than 5% of the tumors are wholly extraosseous; these present most often in the anterior gnathic regions.[5]

The tumor manifests considerable histologic variation, attributed by some to a passage through developmental stages.[5] The variations include both the epithelial and the extracellular constituents of the tumor (Fig 2). The usual appearance is one of sheets of eosinophilic polyhedral cells with well-defined cell borders. Smaller cell nests or clusters and a cribriform arrangement are alternative growth patterns and can usually be found in any given tumor. In addition to the characteristic epithelial component, there is a constant presence of an "amyloidlike" homogeneous ground substance. This is found as calcified or uncalcified concentric Liesegang rings, in irregular deposits, or within the cribriform spaces.[4,5] Prominent intercellular bridges are often a distinctive feature, as is the pleomorphism of the cells and their nuclei. At times, the atypicality suggests a carcinoma, but mitoses are rare to absent. An extreme epithelial variation is a clear cell variant that can simulate either a salivary carcinoma or metastatic renal cell carcinoma.[4,5]

Unlike the circumscribed and often apparently encapsulated adenomatoid odontogenic tumor, the calcifying epithelial odontogenic tumor is expansile and infiltrative. This quality probably accounts for an estimated recurrence rate of 14%.[4,5] There is no recognized malignant variant. Marginal resection with a normal rim of tissue is the recommended treatment.[5]

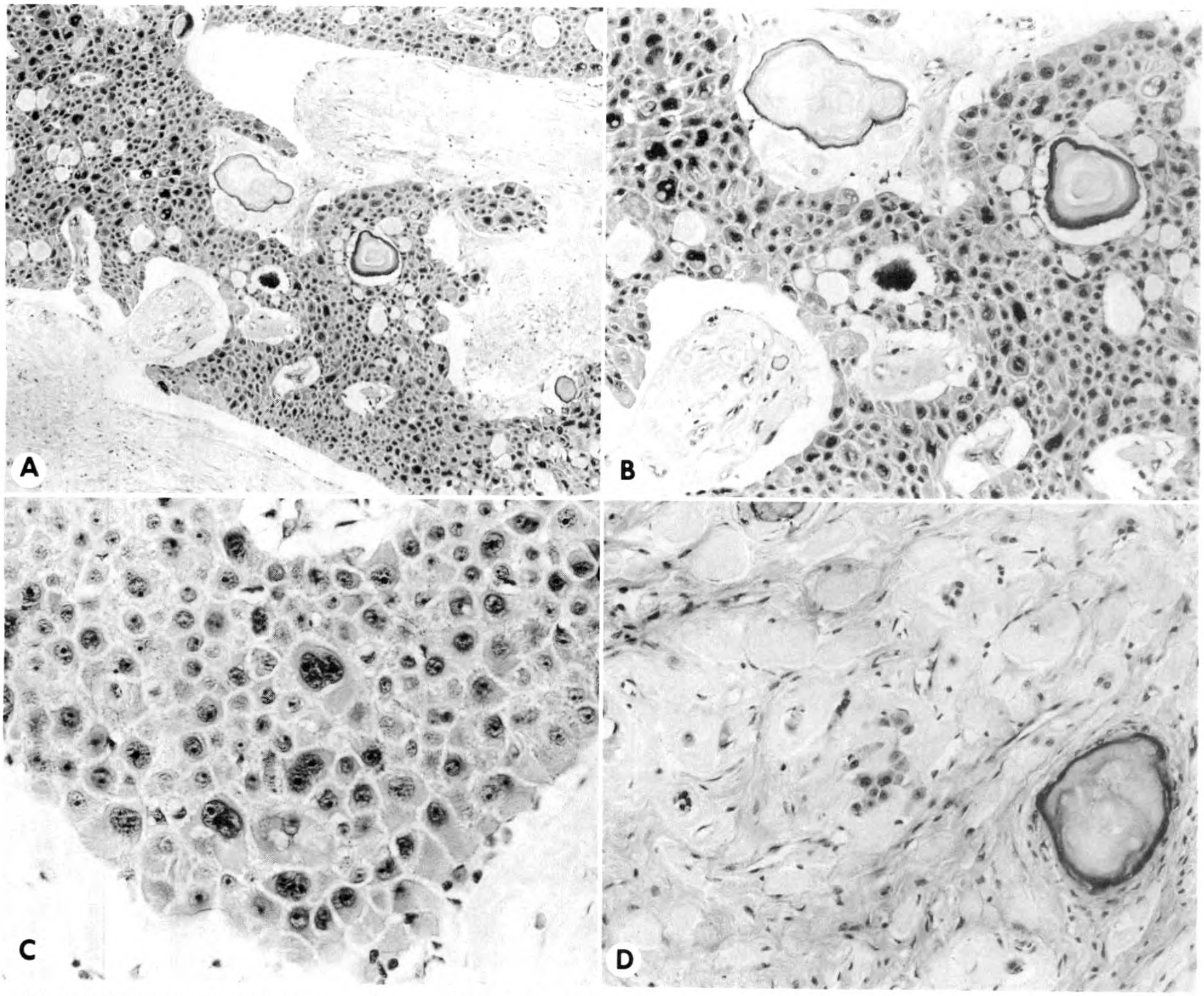

Fig 2. Calcifying epithelial odontogenic tumor (H & E). A) Typical appearance with sheets of eosinophilic, closely apposed cells. Note nuclear pleomorphism and calcified lamellar bodies. B) Higher magnification of field shown in A. C) Squamouslike cells with easily seen intracellular bridges and nuclear pleomorphism. D) Amyloidlike micronodules with entrapped epithelial cells and calcified body.

COMBINED EPITHELIAL ODONTOGENIC TUMOR

This tumor is a hybrid. It consists primarily of areas of adenomatoid odontogenic tumor with foci of calcifying epithelial odontogenic tumor.[6,7] There is a modest predilection for the mandible, and it presents most often in women. The mean age at detection is 19 years.[6,7] Radiographically, the tumor manifests findings of its hybrid components. The biologic behavior is like that of the adenomatoid odontogenic tumor, ie, no recurrences after conservative surgical removal, most often enucleation.[7]

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BOOK REVIEWS

Clinical Anatomy of the Posterior Cranial Fossa and Its Foramina

Johannes Lang. Hard cover, illustrated, indexed, 112 pages, 1991. Georg Thieme Verlag/Thieme Medical Publishers, New York, NY.

The author has provided a purely anatomic text for a very specialized area. All of the eight chapters are important, but the last three are most pertinent to neurotologists: Cerebellopontine Angle, Porus and Internal Acoustic Meatus; Jugular Foramen; and Anatomical Basis for Magnetic Resonance Imaging.

In the Cerebellopontine Angle chapter, there is a disquisition on the internal auditory canal (relationships of the anterior-inferior cerebellar artery and nerves VII and VIII being particularly rich in this discussion). Nine pages making up this chapter contain colorful anatomic structures intermingled by detailed measurements of almost all structures in the posterior fossa.

This is a particularly clinically oriented anatomic text. It is descriptively written and very adequately illustrated. In many ways it is similar to an atlas. The blood vessels were injected to demonstrate arteries and veins. Other tissues, such as connective tissues, appear to have been treated in such a way as to make them stand out. Indeed, many of the anatomic sections are specimens that were photographed in color. However, some of the drawings are involved with miscellaneous data, some of which are not pertinent, including such information as the size of the same structure in the infant, an older child, and an adult, as well as distances from one structure to another.

Compared to other books of neuroanatomy, this text presents an extraordinarily vivid impression of the posterior cranial fossa. The Thieme Medical Publishers have previously published several volumes that are similar, and they are to be commended for this. It is a must for the shelf of the neurotologist.

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Neurobiology of Hearing: The Central Auditory System

Richard A. Altschuler, Richard P. Bobbin, Ben M. Clopton, and Douglas W. Hoffman, editors. Hard cover, illustrated, indexed, 512 pages, 1991. Raven Press, New York, NY.

This is the second book in a neuroscience series that covers the transmission and processing of hearing impulses traveling between the cochlea and the brain. It is not dependent on the first book in the series, which was on the cochlea and published in 1986. The two volumes were designed to augment each other in presenting an understanding of the neurobiology of hearing. The editors offer no explanation for the time that elapsed between the two volumes.

This volume concentrates on experimental data derived primarily from animal studies, and is well referenced. If the reader's patience and concentration last through the myriad of anatomic terms and abbreviations in the first three chapters, in which the auditory pathways to and from the cochlea and cochlear nucleus are reviewed, the data in the remaining 15 chapters will be found to be well organized. The scientific discussions of the biochemical and cellular aspects of pharmacology, neurotransmitters, development, and plasticity should be of interest to the research-oriented otolaryngologist.

Information related to certain endogenous neurotransmitters in the hearing mechanism has been available for some time. This text provides an overview of reasons to use that information, such as current studies of an extremely small subset of neurotransmitters. Immunochemical approaches used to study the wide variety of peptide and low-molecular weight nerve transmitters are discussed in depth. This is significant because neuro-immunology, and especially the use of monoclonal antibodies, has only recently found application in the field of otoneurology. Indeed, neurochemistry, especially neuro-immunology, looms as the cutting edge for potential medical breakthroughs. The effects of known pharmacologic agents such as neurotransmitter agonists, antagonists, and uptake blockers that can be examined in physiologic and behavioral tests is an example of the information made available in this book. It does not take much imagination to foresee the role of neurotransmitters in identifying aging processes, communication disorders, and other medically related phenomena.

Certainly, clinicians who have been separated from their training for a number of years need to be made aware of the advances being made by studies such as those reported in this text, but not necessarily in the depth they are presented. Such technical information may, however, act as a catalyst for further research and application by the investigative researcher.

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When Your Child Is Deaf — A Guide for Parents

David M. Luterman with Mark Ross. Soft cover, 182 pages, 1991. York Press, Parkton, Md, $17.95.

This eight-chapter book is, for the most part, an excellent general guide for the parents of hearing-impaired children. The authors devote the first four chapters to the effects of the hearing loss on the child and his or her family. This is extremely well done by citing useful experiences dealing with frustrations, guilt, anxiety, and the child's loss of self-esteem generated by a poor performance level. David Luterman's experience in this field has endowed him with psychologic insights that he shares with the reader as useful information.

The second half of the book was mostly written by Mark Ross, who begins it with a comprehensive review of audiometric procedures and amplifying systems. Dr Ross admits to being reticent to discuss educational options available for hearing-impaired children, and this is understandable since not every method is suitable for every hearing-impaired child. With that fact in mind, a guide for parents of a hearing-impaired child should present a well-balanced description of all methods without expressing prejudices or preferences — a goal Dr Ross achieved for the most part. However, this section would have been enhanced greatly had he included scientifically based criteria, such as reading levels and speech skills, that parents could use to intelligently select the method most appropriate for their child.

As a whole, this book is quite good. However, the service it will provide parents is limited by what seems to be the educational philosophy of at least one of the authors that all hearing-impaired children will be functional only in a hearing-impaired community.

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MANAGEMENT OF AIRWAY OBSTRUCTION IN PATIENTS WITH CONGENITAL HEART DEFECTS

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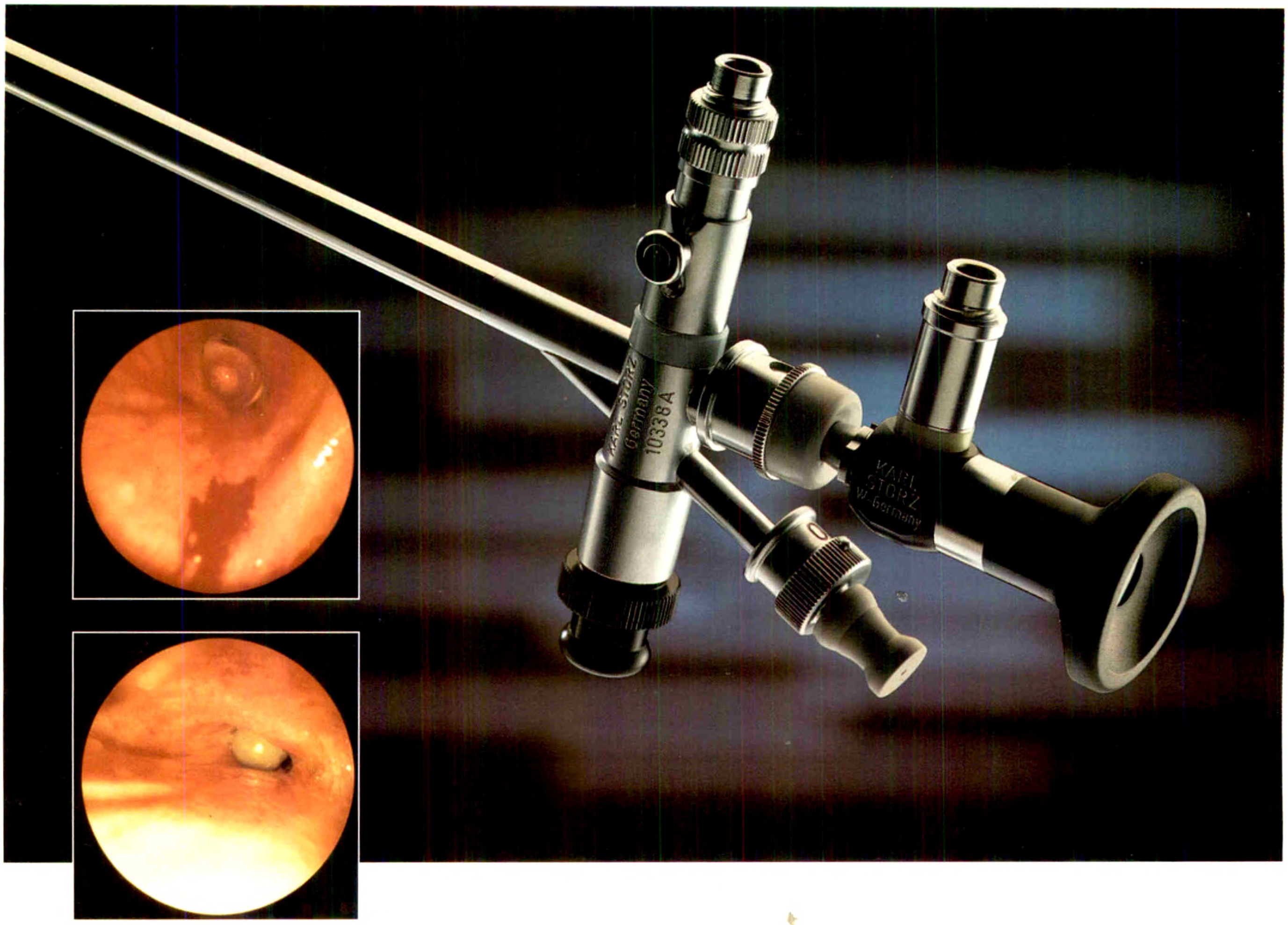

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MANAGEMENT OF AIRWAY OBSTRUCTION IN PATIENTS WITH CONGENITAL HEART DEFECTS

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Airway obstruction may complicate the course of infants undergoing repair of congenital heart disease. Airway obstruction was encountered in seven patients following surgery for complex congenital heart defects (two with interrupted aortic arch, one with pulmonary atresia and a ventricular septal defect, one with tetralogy of Fallot and an abnormal left anterior descending coronary artery, one with truncus arteriosus, and two with complex univentricular heart). In four patients, a conduit was implanted at the time of surgery. In all cases, bronchoscopy accurately demonstrated the cause of the airway obstruction. Two infants had hypoplasia of the left bronchus, and five had tracheobronchomalacia associated with extrinsic compression of the airway by a great vessel or conduit. All were treated conservatively with therapeutic bronchoscopy, tracheotomy, and/or stenting with prolonged mechanical ventilation. Follow-up ranged from 2 to 40 months. Four infants have been weaned from mechanical ventilation and decannulated; two are awaiting decannulation; and one was decannulated and died awaiting surgical repair. To reduce morbidity in infants undergoing surgery for congenital heart disease, the presence of preoperative airway anomalies should be sought and surgery planned to avoid airway compression. Conservative management using diagnostic and therapeutic bronchoscopy, tracheotomy, and stenting together with prolonged ventilator care is rewarding.

KEY WORDS — airway obstruction, congenital heart defects.

INTRODUCTION

Tracheobronchial obstruction is associated with significant morbidity and mortality in patients undergoing repair of congenital heart defects. Although vascular rings are a well-known cause of airway obstruction,[1] only recently has the association between more complex congenital heart malformations and airway compromise been recognized. We have recently reviewed our experience with seven consecutive patients in whom severe tracheobronchomalacia or bronchial obstruction was encountered following repair or palliation of complex congenital heart malformations. Our findings and management of these patients are reported in the present study.

PATIENTS AND METHODS

From August 1988 to January 1991, seven infants (age range 2 days to 7 months) presented with major airway obstruction following palliation or repair of complex congenital heart malformations (see Table). All patients failed extubation following successful palliative or reparative cardiac surgical procedures. Diagnosis was made with bronchoscopy in all.

Anesthesia for bronchoscopy was induced with an inhalational agent or intravenous narcotic depending on the severity of the underlying cardiac disease. All patients were intubated with appropriate-size uncuffed endotracheal tubes and had an audible leak around their endotracheal tube of less than 30 cm H_2O. Through the indwelling endotracheal tube, either mechanical or hand ventilation was maintained. Pulse oximetry and end tidal carbon dioxide measurements were continuously monitored. Spontaneous ventilation was used if vocal cord function was to be evaluated. Immediately before bronchoscopy, a muscle relaxant was given and hyperventilation with 100% oxygen was performed. The airway was evaluated endoscopically by direct laryngoscopy and bronchoscopy using an apneic technique with the aid of Hopkins rod telescopes. A Storz bronchoscope was used to dilate bronchi that were hypoplastic or compressed. After bronchoscopy, the endotracheal tube was replaced, anesthesia was discontinued, and the patient was transported back to the intensive care unit.

Once thoracotomy or sternotomy incisions were healed, the patient was returned to the operating room and a tracheotomy was performed. The average length of time between the cardiac procedures and the tracheotomy was 2 weeks. Conservative management

---

From the Pediatric Heart Institute, Temple University School of Medicine (Davis, Russo), the Departments of Anesthesia and Critical Care (Davis) and Surgery (Tucker, Russo), St Christopher's Hospital for Children, and the Division of Otolaryngology, Albert Einstein Medical Center (Tucker), Philadelphia, Pennsylvania.

Presented at the meeting of the American Laryngological Association, Waikoloa, Hawaii, May 4-5, 1991.

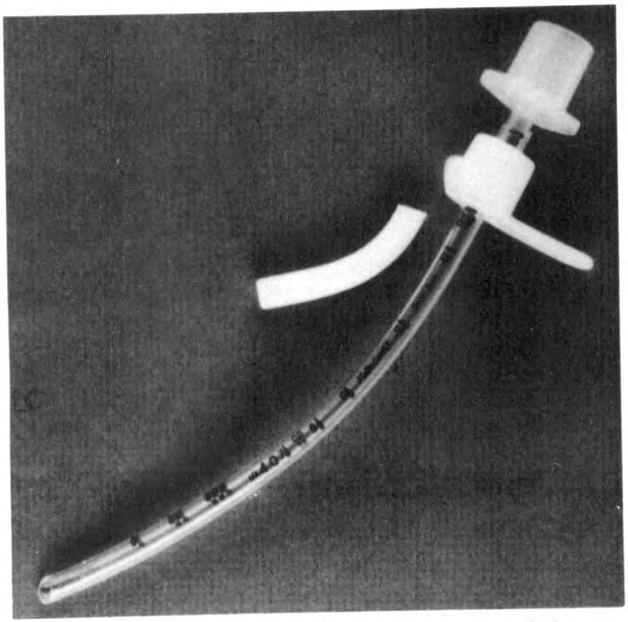
REPRINTS — Deborah A. Davis, MD, Dept of Anesthesia and Critical Care, St Christopher's Hospital for Children, Erie Ave and Front St, Philadelphia, PA 19134.

CARDIAC PROCEDURES AND AIRWAY OBSTRUCTION IN SEVEN INFANTS

Patient	*Age*	*Diagnosis*	*Cardiac Procedure*	*Airway Obstruction*	*Follow-up*
1	Newborn	IAA, VSD	Arch repair by end-end anastomosis, VSD repair	Tracheobronchomalacia, small left bronchus	Alive, good condition (16 mo)
2	Newborn	IAA, VSD	Arch repair by end-end anastomosis, PAB	Tracheomalacia (lower), compression left bronchus	Alive, good condition (36 mo)
3	Newborn	AVC, PA, TGV, HLV	Central shunt, RMBT shunt, hemi-Fontan	Stenosis left bronchus	Alive, good condition (32 mo)
4	Newborn	DORV, HLV, PA, TGV	LMBT shunt, aortopexy	Anterotracheal compression, compression left bronchus	Alive, good condition, awaiting Fontan (18 mo)
5	Premature newborn	Truncus arteriosus type II	Bilateral PAB	Compression trachea	Died awaiting cardiac repair (2 mo)
6	Newborn	TOF, PA, VSD	Complete repair RV-PA homograft	Compression left bronchus	Alive, good condition (40 mo)
7	Newborn	TOF, anomalous LAD	Complete repair RV-PA homograft	Compression trachea	Alive, good condition (6 mo)

IAA — interrupted aortic arch, VSD — ventricular septal defect, PAB — pulmonary artery banding, AVC — atrial-ventricular canal, PA — pulmonary atresia, TGV — transposition of great vessels, HLV — hypoplastic left ventricle, RMBT — right modified Blalock-Taussig, DORV — double-outlet right ventricle, LMBT — left modified Blalock-Taussig, TOF — tetralogy of Fallot, RV-PA — right ventricle to pulmonary artery, LAD — left anterior descending coronary artery.

involving prolonged mechanical ventilation with positive end-distending pressure was applied. Pulmonary function tests were performed in all patients in order to establish the degree of ventilatory support, particularly the optimal positive end-expiratory pressure, necessary to maintain patency of the central and peripheral airways.



No. 3 pediatric Shiley tracheotomy tube modified to function as collar coupled with No. 3 pediatric endotracheal tube. Latter may be cut to any predetermined length.

In patients with bronchial compression (patients 1, 3, 4, and 6), frequent (initially weekly) bronchoscopy with dilation was necessary to avoid lung collapse. Subsequently, bronchoscopic dilation and lavage were performed at monthly intervals for a period of 6 months to avoid lung collapse.

Bronchoscopy was used to evaluate patients with generalized and diffuse airway obstruction (patients 2, 5, and 7) that revealed an extrinsic compression by a vascular structure. Based on the functional dimensions of the airway and degree of mucosal healing, plans for weaning from mechanical ventilation and eventual decannulation were made. Patient 4 had undergone a left modified Blalock-Taussig shunt (a 5-mm Goretex interposition graft between the left subclavian artery and left pulmonary artery) to increase pulmonary flow. Angiography revealed a dilated ascending aorta, and the patient required an aortopexy to relieve the anterior tracheal compression. He continued to require bronchoscopic dilation of his left bronchus for at least 6 months following the aortopexy. His airway was stented to the level of the left bronchus with a specifically calibrated tracheotomy tube fashioned for his particular airway dimensions (see Figure).

Follow-up has ranged between 2 and 40 months in all patients. Six patients were alive and in good condition at last follow-up. One patient died awaiting his cardiac repair.

Four patients have been successfully weaned from mechanical ventilation and decannulated. Patient 1 was decannulated and was free of symptoms after 16 months. Patient 6 was decannulated after 40 months. Patient 3 was successfully decannulated after his second cardiac operation, but required a new tracheotomy and continuous positive airway pressure following his third intracardiac procedure. He is progressing well, although his pulmonary compliance was reduced at his 32-month follow-up visit as compared to the preoperative period. Patient 5 was decannulated 2 months following bilateral pulmonary artery bands and tracheotomy. We believe that a reduction in pulmonary blood flow following the banding procedure reduced the degree of tracheobronchial compression, permitting a successful decannulation. This patient experienced sudden death while awaiting his intracardiac repair. Permission for a postmortem examination was not granted.

Patient 4 is awaiting decannulation at his 18-month follow-up. Patient 7 is awaiting decannulation at his 6-month follow-up.

Patients 1, 3, 4, 6, and 7 were enrolled in our home ventilator care program and were initially discharged home on mechanical ventilation and weaned from that mechanical ventilation at home. In the remaining patients, social and environmental reasons prevented their enrollment in our home ventilator care program. Patients awaiting decannulation were followed up with bronchoscopy and pulmonary function tests at regular intervals. Echocardiography and cardiac catheterizations were performed in all patients between 2 and 24 months following cardiac surgical procedures in order to evaluate the results of the cardiac palliation or repair.

DISCUSSION

The failure to wean a child from mechanical ventilation following a cardiac surgical procedure can be a relatively common occurrence, requiring a thorough evaluation of multiple and possibly interrelated causes. In those patients with elevated pulmonary blood flow, one needs to discern if the problem is related to direct vascular compression of the tracheobronchial tree or is caused by volume overload, interstitial edema, or decreased pulmonary compliance. If there is direct compression of the airway, the compromise is far more life-threatening in the pediatric population than in the adult. In addition to proportionally reduced dimensions,[2] structural features make the infant airway more vulnerable to extrinsic compression. The glottis in infancy has an anterior-posterior dimension of 7 mm and a posterior transverse dimension of 4 mm. Mucosal edema of 1 mm will create a glottic space 35% of normal.[3] The subglottic anterior-posterior diameter, the limiting dimension of the infant larynx, is approximately 5.7 mm at birth.[4] In infants, the cartilaginous, muscular, and elastic supporting structures of the airway are more pliable and susceptible to extrinsic compression. Air trapping and atelectasis are far more common in infancy as a consequence of these anatomic characteristics.

The infant or young child has high peripheral airway resistance, lending a greater tendency to airway closure. Resistance to airflow through the bronchial lumina is proportional to the radius of the lumina; consequently, a small amount of edema, mucus, or compression will markedly narrow the infant bronchus.[5]

It is not surprising, then, that complex cardiac malformations can compromise the infant airway by direct compression (heart or great vessel) or by increased pulmonary artery pressure and pulmonary interstitial edema.[6,7] Cardiac surgical procedures involving prosthetic devices or conduits may result in direct compression of the airway. In addition, pulmonary artery homografts or large systemic to pulmonary artery shunts, by increasing pulmonary blood flow, can distend the pulmonary arteries, which can compress the tracheobronchial tree. Surgical repair of an interrupted aortic arch or severe coarctation of the aorta may affect the tracheobronchial tree if an end-to-end anastomosis is done at the level of the left bronchus. Surgical dissection of vascular structures can result in edema that distorts the anatomic relationships around the tracheobronchial tree. Another inadvertent consequence of this dissection may be recurrent laryngeal nerve or phrenic nerve damage that may not cause airway obstruction, but should be considered in the difficult-to-extubate patient. In the postoperative course, recurrent tracheal infections may lead to subglottic inflammation and stenosis that may be another source of airway compromise.

The workup for potential airway problems should ideally precede the surgical period, as there are well-known associations between cardiac malformations and tracheobronchial abnormalities. There are those centers that use tracheography as a means of evaluating the airway. At our institution, we hesitate to use this method, as it results in inspissated tracheal secretions and bronchospasm despite aspiration of most of the dye after the study has been performed.

We believe that bronchoscopy has several diagnostic and therapeutic advantages. It provides a more dynamic picture of the airway together with a complete anatomic view of the central and peripheral bronchi; it allows ready removal of secretions, lavage, and dilation of obstructed airways; it can be repeated at short intervals; and it facilitates the insertion of internal stenting, as in patient 4.

Surgical methods used to treat intrathoracic airway obstruction with or without malacia include tracheal bronchotomy with patch reconstruction and tracheal bronchial resection by end-to-end or end-to-side direct anastomosis. However, these methods very often require cardiopulmonary bypass and/or circulatory arrest with full heparinization. Bleeding, infection, and dehiscence of suture lines are problems associated with surgical resection and reconstruction of airways with cardiopulmonary bypass. In addition to high morbidity, mortality is as high as 40% in patients with complex congenital heart defects undergoing surgical repair of airway obstruction.[8]

In the present series there was only one death, not directly related to airway obstruction. This occurred in a premature baby who died suddenly with an arrhythmia while awaiting intracardiac repair.

In summary, we believe that tracheotomy, repeated bronchoscopy with or without stenting, and prolonged positive pressure ventilation is a valid alternative to surgical correction of an obstructed airway. The results of this series are rewarding and indicate that a more conservative approach as in this group of patients is warranted.

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NEW PERSPECTIVES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

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THYROARYTENOID INTRAMUSCULAR PRESSURES

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The aim of this research has been to investigate the measurement of laryngeal intramuscular fluid pressures as a technique for assessment of laryngeal muscle activity for diagnostic or experimental purposes. Simultaneous measurements of contractile force, intramuscular fluid pressure, and electromyographic signals were made under isometric conditions in the vocal fold muscles of anesthetized dogs during supramaximal stimulation of the recurrent laryngeal nerve. Covariation of force and pressure was studied in twitch and tetanic contractions, over a range of stimulation frequencies. Tetanic pressure rises were usually smaller than in human limb muscles, but were of sufficient magnitude that effects on blood flow and muscle metabolism are probable. Analysis of relations between pressure and force records demonstrated that intramuscular pressure variations correlated closely to isometric force in their time course. This finding suggests the potential significance of this measure as a relatively nonintrusive indicator of the level of force of muscles involved in laryngeal function, and as a physiological measure of importance.

KEY WORDS — intramuscular pressure, larynx, muscle, vocal cord.

INTRODUCTION

The function of laryngeal muscles has usually been assessed by either electromyography (EMG) or optical techniques. Electromyography is a complex technique,[1,2] and the relation between the force output of muscles and the EMG signal in anisometric contractions is not straightforward in practice.[3] Although optical techniques have been continually improved, they have rarely been applied in a manner that would realize their inherent promise of quantification, and at present it is unlikely that optical findings can be interpreted in a manner that will make possible specification or quantification of the active muscular forces, despite the step in this direction taken by Broad.[4] Measurements of mechanical activity of laryngeal muscles have been rare, and most often they have been conducted in acute experiments, carried out by procedures that do not allow experimental designs with measures repeated on successive occasions, and cannot be used on humans. In the conclusion of a previous study of the effects of reinnervation on the contractile properties of the thyroarytenoid muscle,[5] we noted how desirable would be alternative forms of mechanical measurement that would make possible successive measures of the mechanical response of laryngeal muscles so that, for instance, the time course of recovery after surgery could be evaluated more systematically. In this paper a different procedure will be demonstrated that to our knowledge has not yet been applied to muscles in the cranial nerve area, based on the relation between the variation of intramuscular fluid pressure and isometric muscle force. The present paper will emphasize methods and illustrative findings.

Most previous research on laryngeal muscle mechanics has depended on a one-dimensional conceptualization of muscle contraction as resulting only in longitudinal tension. Nevertheless, reminders of the three-dimensional character of muscle activity have cropped up occasionally for hundreds of years, as appears in the overviews by Partridge and Benton[6] and Reichel,[7] although formalization of three-dimensional analyses of muscle mechanics is relatively recent.

The first three-dimensional physiologic properties of laryngeal muscles to be studied were changes in the transverse viscoelastic properties of muscle during its contraction. These alterations have been long noted in the general literature on muscle mechanics. The effect has been noted since Leonardo da Vinci and has been used experimentally since the late 19th century.[6,7] The first quantitative study of laryngeal muscle mechanics known to the authors, by Réthi[8] in 1897, was based on inference of canine thyroaryte-

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noid muscle force from changes in transverse elasticity in vivo. Mårtensson and Skoglund[9] based observations of speed of laryngeal muscle contraction on the muscles' lateral displacement during contraction; Haji et al[10] have studied the changes of transverse viscoelasticity of the vocal folds during thyroarytenoid contraction; and Berke et al (unpublished observations, 1990) have more recently studied the effect of contraction on the transverse elasticity of this muscle in vivo.

In 1948, Hill[11] demonstrated an increase of intramuscular fluid pressure on the order of 200 mm Hg in frog gastrocnemius muscle during tetanic contraction. Discussing the study later in his autobiographic survey of his own and related physiological research,[12] he saw its main implications as explaining both the small reversible volume changes found in this muscle during contraction, and the effect of high levels of contraction in the leg in ceasing blood flow. He was able to verify computationally that if the bulk elasticity of muscle is assumed to be equal to that of water, the measured fluid pressure changes do approximately account for the observed volume changes of contracting muscle. Nevertheless, other explanations have been proposed for rapid volume changes that are approximately synchronous with contraction.[13] Volume changes that persist after relaxation are attributed to metabolic processes. However, Hill[11] doubted that these pressure changes would be found in muscles with straight parallel fibers, such as the frog's sartorius, although he expected them to be general in other muscles of complex fiber course similar to the gastrocnemius. His prediction would thus also extend to the thyroarytenoid muscle, in which at least a large proportion of the fibers appear to be parallel, although there are significant findings indicating a more complex structure.[14]

Needham[13] has surveyed the various contributions to the analysis of volume changes, especially from Ernst, Meyerhof, and Abbott and their associates. An important question was raised by early studies of Meyerhof,[15] who found that volume changes were much smaller in lightly loaded isotonic contractions than in isometric contraction, while they increased with the level of load. Hill[11] attributed this to the hypothetic development of smaller fluid pressures in isotonic contraction. Fluid pressure measurements with change of muscle length are difficult to make reliably today, as they were in Hill's day; consequently, the available findings still come primarily from isometric studies. However, the eventual situations of interest also involve anisometric contraction, so that the effects of both stepwise and continuous changes of muscle length must be studied.

The volume and static pressure of interstitial fluid within muscles result from movement of fluid from the capillaries, its resorption by them, and lymph flow. The components of this balance are summed up in the well-known Starling equation. Hargens[16] provides details of the bases of the generation of this pressure. Interstitial fluid volume of skeletal muscle varies from about 8 to 20 mL per 100 g wet tissue[17]; thus, it amounts to a quarter or less of the total fluid in skeletal muscle, which in turn composes a mean of 79.2% of the weight of skeletal muscle.[18] However, this volume is variable: alterations of interstitial fluid pressure can considerably affect the rate of exchange of fluid between the circulating blood and the interstitial space.[19]

In recent research, the relationship of intramuscular fluid pressure to other physiological quantities has been studied primarily within the orthopedics and biomechanics literatures, and the in vivo application has been primarily in large limb muscles, except for occasional studies in small animals. An important series of studies has clarified the relationship between the interstitial fluid pressure of muscle and isometric force of skeletal muscle.[20] However, at a given level of contraction, fluid pressure varies from place to place within the muscle[21-23]; these relationships are not arbitrary, but at present they are incompletely investigated. There may be found a positive gradient of static fluid pressure as one passes from peripheral to deep tissues within the muscle, depending on whether the boundaries of the muscle are rigid or not.[20] Intramuscular pressure also increases with externally caused longitudinal tension of the muscle.[24] Detailed effects of these factors vary depending on the anatomy of the particular muscles in question.

Apart from these complicating factors, it has been found that interstitial fluid pressure at a given point is highly linearly related to isometric muscular force,[21,23,25] although the slope of this relation cannot at present be predicted. Although local fatigue probably develops where pressures exceed 20 to 30 mm Hg, intramuscular pressure in isometric contraction may reach levels as high as 600 mm Hg in maximal contraction.[20,26,27] Intramuscular pressure is more closely related to force than is the EMG signal, which is usually compared to force in rms or rectified and smoothed form.[25,28] The relation between intramuscular pressure and force, being purely mechanical, is not altered under conditions of fatigue or at high levels of contraction, as is the relation of the EMG signal to isometric force.[3,25]

METHODS

The dog was chosen for initial experiments because of the availability of a reasonable fund of information on the structure[29,30] and physiological properties of active canine vocal fold muscle both from earlier published in vivo results[9,31-35] and from our own in vivo research.[5,36] The available animals were small, of mean weight 6.7 ± 0.5 kg.

The experiments were approved by the USC IACUC. The methods were similar to those described earlier,[36] with the addition of the measurements of intramuscular pressure. Seven dogs were studied under general anesthesia. Each animal was premedicated with atropine 1/120 grain, ketamine 100 mg, and acepromazine 5 mg. General anesthesia was induced with pentobarbital. The animals were immobilized in the supine position on the operating table. After anesthesia was induced, the animals were intubated and assisted ventilation was begun.

The anterior neck and upper chest were shaved, prepared, and draped. A 6-cm midline vertical incision was made from just above the hyoid bone to the sternal notch. The median raphe was divided and the cartilaginous structure of the larynx and upper trachea was exposed. The hyoid bone was freed of the suprahyoid and infrahyoid musculature. The recurrent and (if present) pararecurrent laryngeal nerves were identified bilaterally. The trachea was transected at the fifth ring, and the animal was reintubated distally. The proximal portion was fixated to prevent laryngeal movement during respiration. Supramaximal stimulation of the recurrent nerve was used to activate the muscle.

The cricothyroid space was entered in the midline and a precise laryngofissure was created. Identification of the motor portion of the caudal laryngeal nerve was confirmed bilaterally by electrical stimulation. Each hemilarynx was now a separate experimental preparation.

For the study of a given muscle, a square of cartilage directly anterior to the thyroarytenoid muscle was created by incision through the outer perichondrium, the cartilage, and the inner perichondrium. The mucosa was freed inferiorly and superiorly. A suture of 2-0 polyester was placed between the cartilage and the inner perichondrium. The other end of the suture was attached to it. The EMG electrodes were placed directly in the thyroarytenoid muscle, and a 1-mm incision was made in the anterior aspect of the muscle for placement of the pressure transducer. The transducer was then gently threaded about 5 mm into the muscle and finally withdrawn 1 mm to allow a small fluid collection anterior to the transducer tip; thus, the transducer monitored fluid pressure. The arytenoid cartilage was fixated by pinning it to the posterior section of the cricoid cartilage, and this pin was fixed externally, so that muscle contraction was isometric and other muscles could not affect the measurement of thyroarytenoid muscle activity.

As experimentation proceeded, the animal was given maintenance doses of 5 mg/kg of pentobarbital as needed for adequate anesthesia. At the culmination of experimentation, the animals were given lethal doses of pentobarbital (100 mg/kg).

The EMG signal was recorded differentially from fine wire electrodes inserted separately into the vocal fold muscle with the interelectrode axis parallel to the muscle fibers; the ground electrode was attached to the edge of the neck incision. The capacitive force transducer had previously been calibrated with weights. Intramuscular fluid pressure was sensed by a transducer of 1.3 mm diameter (Camino Laboratories model 110-4D) at the end of a flexible fiberoptic cable inserted into the muscle. The principle of this transducer is discussed by Cobbold[37]: light is transmitted down one fiberoptic bundle to the tip, and the displacement of a diaphragm by external fluid pressure determines the amount of light reflected back through a second detector bundle. This transducer had been previously calibrated. Pressure measurements were made close to the center of the vocal fold, but there was some inevitable variation of transducer position across animals. Both mechanical transducers were linear over the range of interest. All these signals were recorded on a computer at not less than 5,000 points per second, together with a timing signal from the stimulator (Grass S88) that occurred simultaneously with the stimulation of the recurrent nerve by 0.1-millisecond supramaximal pulses presented through a stimulus isolation unit (Grass SIU7).

RESULTS

The intramuscular fluid pressure measured in resting thyroarytenoid muscle at resting length (fold adducted) had an average value of 14 mm Hg in a series of data sets across all animals studied. Since resting pressures were sometimes affected by preceding contractions, this value is a super-mean from the means for individual animals from the first set of pressure measurements on a given day for the vocal fold in question. Mean values for individual animals ranged from 1.7 to 30.9 mm Hg. Negative pressure values were rare, but small ones were observed.

Observations were made in three animals of the effect of change of muscle length on resting intramuscular pressure, over a range of 4 mm extension. Possibly, small changes of transducer position during extension of the muscle perturbed these measurements. If only data sets in which the relation was monotonic or nearly so ($r = .86$) are included, there was a mean increase of intramuscular pressure of 4.4 ± 3.3 mm Hg per millimeter of extension.

At times a slow drift of fluid pressures was observed across successive activations of the muscles. For instance, a progressive decrease in resting fluid pressure from 21 to 13 mm Hg was observed over a series of five activations of the thyroarytenoid muscle of one animal. These values did not reflect a calibration drift in the pressure transducer, which was stable over the time intervals in question when tested in a water column and shows a maximum zero drift of about 3 mm Hg per 24 hours. Probably these changes reflected small shifts of fluid within the muscle occurring as a result of inequalities of local fluid pressure during contraction (Darcy's law effects).

The force range to be considered is from resting state to full tetanic contraction, which is usually, although not always, approximately equal in force to maximum voluntary contraction.[38] Thus, one focus of our initial studies has been on the onset of tetanic contraction, which permits a contrast of pressure and force extremes in resting and tetanic muscle. These data were available for five animals. When the recurrent laryngeal nerve was stimulated at 80 to 240 pulses per second, the mean tetanic pressure rise in this data set was 44.1 ± 36.5 mm Hg, varying widely from less than half this amount to more than twice it. The observed variability is accounted for partly by an increase of intramuscular pressure with activation rate over this range, and partly by cross-subject variability in the relation between contractile force and pressure as discussed below. Tetanic stimulation often, although not always, produced an increase in intramuscular pressure sufficient to affect the microcirculation.[20,23,26,27]

However, higher increases were observed in the tetanic burst paradigm illustrated below — rises sometimes ranging as high as those observed by Hill[11] in frog gastrocnemius (despite the difference in fiber course noted above), with a range of about 250 mm Hg. It should not be assumed that this is a functional maximum pressure for the muscle, since pressure increases resulting from other sources are also observed, as discussed below.

Previous studies noted above have indicated that the relation between variation of isometric force and intramuscular fluid pressure at a given point is linear.[21,23,25] However, as with isometric EMG, this slope of this relation cannot be predicted at present. In our data, the force-pressure relation was variable across animals. Slopes (Δg/Δmm Hg) determined just after the onset of tetanic contraction varied from subject to subject by a factor of six. (The overall mean for 23 tetanic onsets studied in five animals was 2.094 ± 1.221.) This cross-subject variation has also been observed in experiments on human limb muscles.[27] Some token-to-token variation of slope within a given animal was also observed, but some findings suggested that it may be possible to design procedures to obtain reliable data. For example, if the first data point is omitted in a series of tetanic rises (stimulus rates ranging from 80 to 240 pulses per second) for the seventh dog, the mean slope for six onset maneuvers is 0.739 ± 0.077. If it is included, the mean slope for the seven onsets is 0.986 ± 0.656. Future research must investigate the prediction of the force-pressure relation across subjects, the bases of its short-term variation within subjects, and the effects of alterations of muscle length on this relation.

The Figures illustrate the temporal relation of the pressure and isometric force records for sample data from the last animal studied, which was small (6.3 kg). The data shown come from a transducer placement at the approximate center of the muscle. The time course of pressure variation in recordings from other transducer locations, such as directly beneath the mucosa, differed from that of central pressures, with potentially important physiological implications. Apart from rescaling from electrical to mechanical units, the data have been left in the form in which they were recorded.

Figure 1A shows the timing of the stimulator pulse, in the illustration of an isometric twitch. Figure 1B shows intramuscular fluid pressure. The peak pressure rise here is just over 23 mm Hg. The pressure signal is updated every 900 microseconds; the resulting high-frequency component in its signal may be ignored for physiologic interpretations. Figure 1C shows isometric force with the DC offset corresponding to resting tension set to zero. The pressure signal reflects the limited system bandwidth of the available system (−3 dB at 33 Hz), and the pressure signal is delayed relative to the force by electronic processing; the lag duration will be specified approximately below. Figure 1D is the evoked EMG signal with an arbitrary scale.

Figure 2 shows the cross-correlation function (Fig 2A) between the pressure record (Fig 2B) and isometric force (Fig 2C), in the response to 50-millisecond repeated bursts of tetanic stimulation at 160 pulses per second. The cross-correlation function plots the degree of scale- and offset-free similarity between these signals as a function of the lag between them. It is used here in order to identify the lag between pressure and force as the point at which the value of the function peaks, since it is known that the physical variation of isometric force and intramuscular pressure is almost simultaneous. Here the cross-correlation function was calculated on the basis of 4,096-

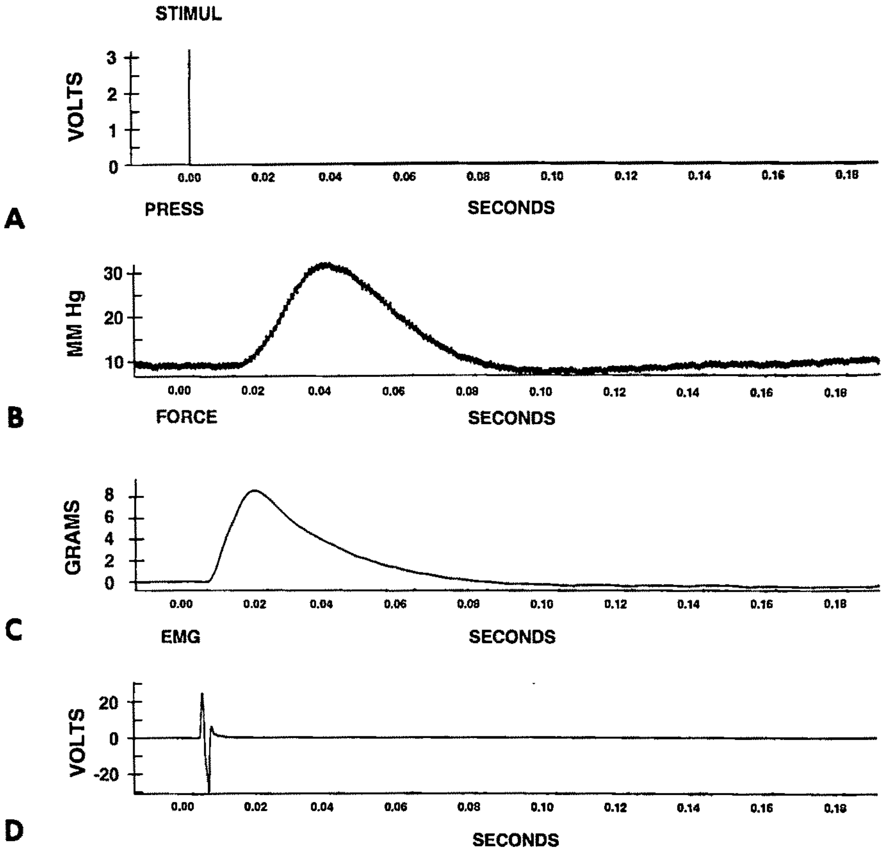

Fig 1. Twitch contraction of vocal fold of dog 7. Force and pressure changes are simultaneous, but electronic processing results in apparent temporal displacement indicated in Fig 2. A) Stimulator timing pulse simultaneous with stimulation. B) Intramuscular fluid pressure. C) Isometric muscle force, with resting tension set to zero. D) Electromyographic signal with arbitrary scale.

point records digitized at 10 kHz, including three cycles such as those illustrated. The cross-correlation function reaches a peak of r = .97 with a 14-millisecond lag between the two records. This delay includes both the processing lag noted above and the filter characteristics of the system; it is almost eliminated in a different version of the measurement system electronics, which was not available to us. The gradual fall of the cross-correlation function reflects a computational convention (addition of zeroes to the first data set) rather than an actual progressive decrease in the agreement between the two signals. In seven such data sets for this animal, the mean peak of the cross-correlation function was r = .939 ± .026. Since these data sets included 28,672 points, this correlation coefficient was significant at better than the .001 probability level.

Figure 3 superimposes the pressure and force records of Fig 2 with elimination of the indicated processing lag and visual rescaling to the same peak-to-peak amplitude. The close relation between the pressure and force signals is clear in both of these data sets.

DISCUSSION

Some remarks on the measurement system are necessary. The pressures are gauge pressures, ie, differences from atmospheric pressure. Above 250 mm Hg, the linearity of the measurement system used in the present study decreases. However, the high correlation (ie, highly linear relation) between force and pressure measures exemplified in Fig 2 indicates that any resulting perturbation in the present study was small. Most pressure values measured fell well below this level, in any case.

A major objective of the present study has been to determine the feasibility of such measurements in small laryngeal muscles. This question has been answered positively in the sense that the measurement of fluid pressures in a muscle that averaged about 1 cm in length in the small animals used made the present study almost a worst case. Since the muscles studied here had about two thirds the length of adult human ones, their volume was probably not more than one third that of the human thyroarytenoid muscle.[39]

Resting intramuscular pressure is expected to be slightly above atmospheric. The observed mean 14 mm Hg elevation of resting pressure is approximately accounted for by estimating the compliance of the interstitial fluid compartment on the basis of measurements from cat and rat muscles, which agree at 1.4 mL/100 g tissue x mm Hg,[19,40] and considering the effect of the introduction of the volume of the transducer (about 5 mm^3) in raising the intramuscular fluid pressure. Thus, the ratio of the volume of the

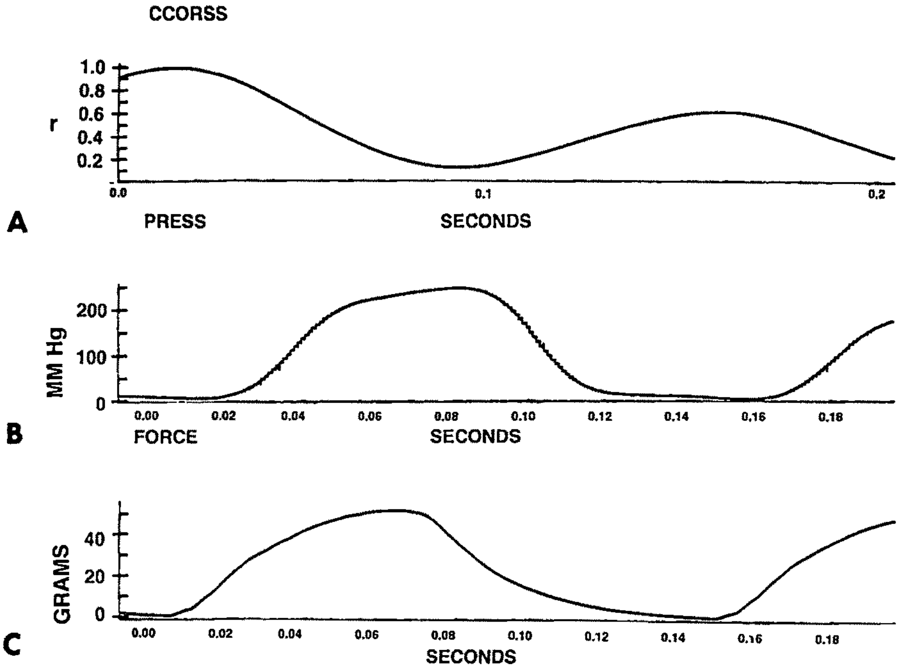

Fig 2. Relation between intramuscular pressure and isometric force in response to 50-millisecond bursts of tetanic stimulation at 160 pulses per second. A) Computed cross-correlation function between B) pressure and C) force records. Here, cross-correlation function reaches peak of r = .97 at 14-millisecond lag, indicating close relationship between pressure and force records when delay introduced by electronic processing in pressure system is taken into consideration.

muscle studied to that of the pressure transducer is a factor limiting measurement accuracy. In a future study, measurement accuracy could be improved by precise monitoring of intramuscular transducer location, by study of larger muscles as in the human larynx, and by decrease of the volume of the pressure transducer.

The fact that measurements of intramuscular fluid pressure could be made within the vocal folds of small animals using a size and type of transducer that still can be improved suggests that application in much larger human subjects need not be remote. The pressure transducer used in the present study was too large (1.3 mm diameter) for routine application in small muscles of human subjects. Foreseeable miniaturization could conceivably reduce such transducers to approximately the size of fine-wire electrodes (circa 50 μm diameter) for EMG; as in EMG, the available procedures for measurement of intramuscular fluid pressure would remain invasive to some degree. However, the pressure measurement is not suggested as a replacement for the EMG signal. The pressure signal, which represents the mechanical result of excitation-contraction coupling, is complementary to the EMG signal, which represents the spread of muscle excitation. Their combined use more adequately represents muscle activity than does either alone.

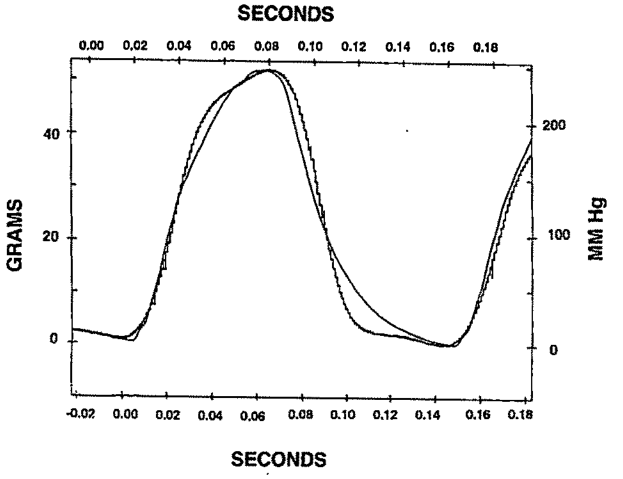

Fig 3. Superimposition of pressure and force records from Fig 2 indicates their similarity. Force record is smooth and pressure record stepped, as in Fig 2. Electronic lag defined by cross-correlation function is eliminated to show physical simultaneity of records, and vertical magnitude scales are equated. Top scale — time base for pressure, bottom scale — time base for force.

The range of intramuscular pressures during contraction observed in the present study is smaller than the higher pressure levels observed in human limb muscles during high levels of contraction (up to nearly 600 mm Hg).[27] One reason may be the fact that the animals and muscles studied were quite small. Inference to absolute pressure values that might be observed in the human vocal folds is thus limited by the fact that the structures studied would be much larger in adult human subjects. It is also the case that the course of muscle fibers in the canine vocal fold, which would affect the pressures developed, is not as well studied as it is in humans.[14] The fact that the mucosa of the canine vocal fold is thicker and somewhat differently constructed[30] from the human one would also affect comparison of the measurements.

The measurement of intramuscular fluid pressure has several distinct types of significance in the study of voice physiology. Part of this significance pertains to the special mechanical situation of the vocal fold. In most muscles, of course, level of muscle activity and muscle length would be the main determinants of the variation of intramuscular pressure at a given point. However, in the vocal folds, not only intramuscular pressures from longitudinal tension,[24] but also collision pressures[41] would sum[42] with the interstitial fluid pressure variation that results from active muscle contraction, in their mechanical effects on tissues and movement of fluids within the vocal fold.

An important consideration is the relation between interstitial fluid pressures and capillary blood pressures. Direct determinations of capillary blood pressure in skeletal muscles of dogs and cats provide values in the range from 20 to 40 mm Hg.[16,43,44] Since blood flow may be intermittent, the quantity is necessarily variable depending on physiologic conditions, and it varies for different observation techniques, but these figures may be taken to provide a rough estimate.

It will be evident that these values are an order of magnitude smaller than those quoted as maximum pressures during voluntary contraction in human skeletal muscle, and even somewhat smaller than the tetanic pressures observed in the canine thyroarytenoid muscle. Consequently, it is not surprising that at high levels of contraction, muscle activity proceeds under ischemic conditions when interstitial pressures become so high that blood flow decreases or is entirely obstructed, resulting in more rapid fatigue than when normal blood flow is preserved.[20,44,45] It has been found that laryngeal muscles possess a strong oxidative metabolism with some specializations that are probably related to the degree of physiologic fatigue resistance found in laryngeal muscles of larger animals.[36,46-48] This is consistent with the respiratory function of these muscles, for which resistance to fatigue would be indispensable for survival. Consequently, the finding of this paper that thyroarytenoid intramuscular pressures resulting from high levels of contraction alone are probably sufficient to obstruct blood flow through the muscle suggests a metabolic significance for laryngeal intramuscular fluid pressures.

The covariation of intramuscular pressure and isometric force in a small laryngeal muscle has been exemplified in the Figures above. Although the variation across different specimens of the homologous muscles is such that complete standardization of this relation across subjects and muscles does not appear likely,[20,27] it is likely that in particular conditions standardization is possible to the degree necessary. This is indicated by the use of measures of interstitial fluid pressure to monitor compartment syndrome.[49] Once this degree of reliability is granted, then this measure makes possible new experimental paradigms. For instance, so far measurements of the effects of laryngeal surgery on the mechanical characteristics of laryngeal muscles have required separation of one end of the muscle from the laryngeal cartilaginous skeleton, which can be done only in an acute operation. However, standardization of measurement of intramuscular pressure would basically require only that the muscle length and approximate transducer location be reproduced at each session. Thus, it would be possible to track changes in muscle function over time, in experimental animals[50] and perhaps eventually in patients. Since the pressure measurement is made within the muscle tissue, it would not have the requirement of surface accessibility of the muscle that is a limitation of techniques based on measurement of the external mechanical properties of the muscle. Thus, despite the complexity of the mechanical determinants of the local intramuscular pressures, further research into the determinants of the scaling of the relationship between pressure and force is merited in order to make more precise the inference of muscle force from intramuscular pressure under specific conditions.

Other questions that must be addressed in further detail are the effects of change of muscle length on intramuscular pressures, further miniaturization of the pressure transducer, and improvement of the dynamic characteristics of the transducer. It appears from our own and previous findings that the relation of muscle length to intramuscular pressure is systematic, although it requires further study in detail. Further miniaturization and improvement of dynamic characteristics are technical problems that are probably solvable. Since the variation of intramuscular fluid pressure is more closely related to the temporal course of skeletal muscle force than is the EMG signal,[25] and intramuscular fluid pressures have important physiological significance in such areas as blood flow and transcapillary fluid balance, the application of this measure for research and clinical measurement of laryngeal muscle function merits further investigation, which should extend to other laryngeal muscles.

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FUNCTIONAL ENDOSCOPIC SINUS TECHNIQUES

A course on functional endoscopic sinus techniques will be held in Graz, Austria, July 1-3, 1993. It will be conducted in German. For further information, contact Prof Dr Heinz Stammberger, University ENT-Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; telephone 43 316 385 3448; fax 43 316 385 3425.

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LARYNGOTRACHEAL RECONSTRUCTION FOR SUBGLOTTIC STENOSIS

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Laryngotracheal reconstruction (LTR) has been employed for the treatment of severe laryngotracheal stenosis for the past 6 years at Johns Hopkins Hospital. Thirty-one children underwent LTR with costal cartilage grafting, 24 of whom had Aboulker stents placed. Short stents were used in 22 patients. Six patients received definitive treatment in a single-stage LTR; 1 child had no stent placed. Twenty-six (84%) of the 31 patients were decannulated. It was concluded that decannulation can be obtained in selected patients with the short Aboulker stent or single-stage LTR. A new classification system for laryngotracheal stenosis, based on objective measurements and the separate analysis of posterior glottic fibrosis, was developed. The proposed classification system allows recommendations for treatment. Moreover, it can be easily reproduced and may facilitate comparison of results.

KEY WORDS — laryngeal stenosis, laryngotracheal reconstruction, laryngotracheal stenosis, subglottic stenosis, tracheotomy.

INTRODUCTION

The widely accepted treatment for laryngotracheal stenosis (LTS) in children unresponsive to endoscopic therapy is laryngotracheal reconstruction (LTR). The technique with costal cartilage grafting, as popularized by Cotton, is used in many centers throughout the world with good results.[1-6] Although intraluminal stents of various materials, lengths, and duration of use have been advocated, the long Aboulker stent with an attached metal tracheotomy tube remains the most popular.[7] A recent modification, the single-stage LTR without an indwelling stent, has been reported.[8-10]

Efforts to compare the reported results of surgical correction of LTS in children are hampered by the lack of a precise, easily reproducible staging system. Cotton's system,[11] which is based on the degree (percentage) of luminal obstruction by the stenosis, continues to be the system most widely used. However, it may be difficult for some to reproduce, and thus may prevent meaningful comparisons between institutions.

This report describes the experience with LTR at Johns Hopkins Hospital, focusing on the use of short Aboulker stents and the encouraging results of single-stage LTR. In addition, a new classification system, based on objective measurements of the lumen, is proposed. This system also incorporates the complicating factor of posterior glottic fibrosis. Morever, this classification is easily reproduced and may also give guidance for therapy.

PATIENTS, MATERIALS, AND METHODS

Thirty-one patients underwent LTR with costal cartilage grafting at Johns Hopkins Hospital between 1985 and mid-1991. Aboulker stents were placed in 24 patients (short in 22 and long in 2). One patient had

TABLE 1. PATIENTS SUCCESSFULLY DECANNULATED AFTER LARYNGOTRACHEAL RECONSTRUCTION

Pt	*Age at Surgery (y)*	*Class*	*Procedure*	*Stent Duration (mo)*	*Time to Decannulation (mo)*
1	10	IP	PG	1	1
2	3	I	AG	1	3
3	4	III	AG/PG	23	7
4	16	II	AG/PG	2.5/17*	7
5	5	II	AG	1.5	2
6	3	II	AG	1.5	1.5
7	3	II	AG	No stent	34
8	3	IP	AG/PG	1	7
9	3	III	AG/PG	13	2
10	2	III	AG/PS	17	25
11	2	II	AG/PG	2	5
12	5	II	AG/PS	2	16
13	3	II	AG/PG	2	1
14	3	I	AG/PS	2	13
15	6	II	AG/PS	2	10
16	3	IIP	AG/PG	1	5
17	3	IIP	AG/PG	1	15
18	2	II	AG/PS	2	3
19	12	IIP	PG	1	1
20	7	II	AG/PG	1	8

PG — posterior costal cartilage graft, AG — anterior costal cartilage graft, PS — posterior cricoid split.

*Long stent broke; replaced with short stent.

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, April 13-14, 1992.

REPRINTS — Bernard R. Marsh, MD, Johns Hopkins Hospital, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 2. PATIENTS WITH TRACHEOTOMY TUBES IN PLACE AT PRESENT

Pt	*Age at Surgery (y)*	*Class*	*Procedure*	*Stent Duration (mo)*	*Comments*
21	6	IP	AG/PG	1	Initially decannulated with normal subglottic size, but tracheotomy done for obstructive sleep apnea (upper airway obstruction)
22	8	II	AG	2	Tracheotomy tube in place for pulmonary toilet after respiratory arrest from long stent–tube occlusion
23	3	III	AG/PS	8	Plugged stent with new suprastomal stenosis that responded to endoscopic therapy; 4-mm airway; final result pending
24	2	II	AG	1	Survived accidental decannulation; decannulation deferred
25	3	III	AG/PG	1	Patient has spondylometaphyseal dysplasia

AG — anterior costal cartilage graft, PG — posterior costal cartilage graft, PS — posterior cricoid split.

no stent. Six patients had anterior cartilage grafts only; 6 had anterior grafts and posterior cricoid splits; 11 had anterior and posterior costal cartilage grafts; and 2 had posterior grafts only. The remaining 6 children underwent single-stage LTR without indwelling stent placement. All 6 of these children had anterior cartilage grafts only.

The characteristics of each patient who had a stent placed (1 without a stent) are depicted in Tables 1 and 2. There were 14 boys and 11 girls. Two patients (8%) had congenital subglottic stenosis, while the remainder of stenoses were acquired. Fourteen (56%) former premature infants and 9 others (36%) had stenosis due to sequelae from long-term intubation. Seven patients had previous procedures at other institutions. The youngest child at the time of reconstruction was 21 months old; the median age was 3 years.

Fiberoptic laryngoscopy was performed, with local anesthesia, to assess vocal cord motility in all patients. Microlaryngoscopy, under general anesthesia, was performed to permit a detailed assessment of the larynx including the posterior glottis. The 2.7-mm and 4-mm diameter Hopkins telescopes were used to determine the size of the lumen; the system used for classifying these data is shown in Table 3. The remainder of the trachea was examined with the pediatric ventilating bronchoscope and telescope.

The surgical procedure described by Cotton and Myer[1] was used with one principal modification: a short (suprastomal) Aboulker stent (see Figure) was used in most patients (22 of 24). These short stents were left in place for 4 to 6 weeks for anterior and/or posterior cartilage grafts. For posterior cricoid division without grafts, the average time of stenting was 2 to 6 months. Short stents were left in place for up to 23 months in patients having no identifiable lumen.

The surgical procedure for single-stage LTR without stent placement, as described by others,[8-10] was used in six patients (Table 4). In each, the preoperative lumen was greater than 2.7 mm in diameter. All children had normal vocal cord motility, and there was no significant posterior glottic involvement. There were four girls and two boys with an average age of 40 months at time of surgery. In four former premature infants (average 30 weeks' gestation at birth), the stenosis resulted from prolonged intubation, and in two other patients it was congenital. Two children required no tracheotomy at all.

RESULTS

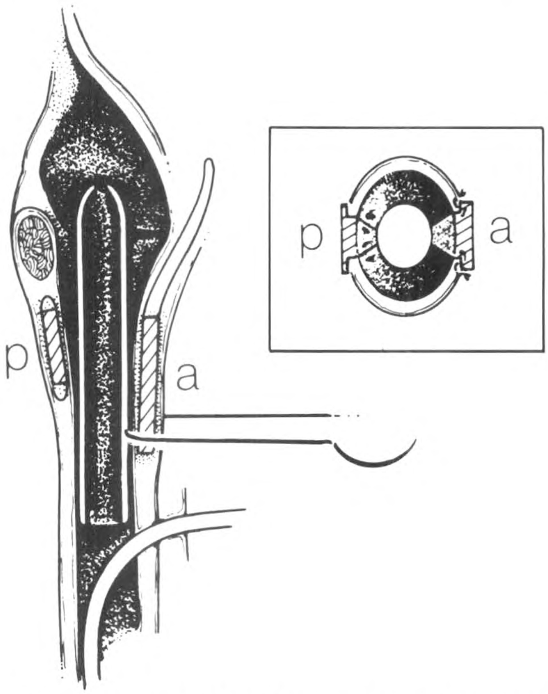
Stenoses were classified as follows: class I (n = 2), class IP (n = 3), class II (n = 12), class IIP (n = 3), and class III (n = 5). In addition, all 6 patients undergoing single-stage LTR had class I stenoses. Twenty (80%) of 25 patients who underwent LTR with stent placement (1 without a stent) were decannulated (Table 1).

Five children still have a tracheotomy tube in place (Table 2). One of these five was initially decannulated, but later required a tracheotomy for obstructive sleep apnea. In one child with spondylometaphyseal dysplasia, LTR did not achieve a satisfactory airway. There were several other complications. Early in this series, a child who underwent LTR with placement of a long Aboulker stent with its wired metal tracheotomy tube sustained respiratory arrest from tube occlusion. After this event, the tracheotomy tube was

TABLE 3. CLASSIFICATION OF LARYNGOTRACHEAL STENOSIS

Lumen Size	*Class*	*With Posterior Glottic Fibrosis**
>2.7 mm	I	IP
≤2.7 mm	II	IIP
No functional lumen	III	IIIP

*With limitation of vocal cord abduction.



Short (suprastomal) Aboulker stent secured by suture. Inset shows anterior (a) and posterior (p) grafts in place.

left in place for pulmonary toilet. The other long stent broke at the wire suture, necessitating its endoscopic removal; a short stent was used to replace it. The patient was later decannulated. One child who underwent an anterior cartilage graft and posterior cricoid split had persistent aspiration with the short stent, and after 1 week the hole in the tip of the stent was plugged endoscopically. After removal of the stent 8 months later (a delay of 3 months due to lack of family compliance), a new stenosis between the inferior portion of the stent and the tracheotomy tube was discovered. This stenosis responded to laser therapy achieving a 4-mm lumen, and the child is awaiting decannulation. All rib cartilage grafts were harvested by the otolaryngology–head and neck surgery team, without complication. Seven children eventually underwent closure of a tracheocutaneous fistula.

Twenty-two children had short stents placed, 18 (82%) of whom were decannulated. The average duration of stent placement, the average time to decannulation, the average number of endoscopic procedures after LTR (not including decannulation), and the number of patients decannulated for each class of stenosis are shown in Table 5.

The six children who underwent single-stage LTR, each of whom had a lumen greater than 2.7 mm without posterior glottic fibrosis (class I), were decannulated at the time of surgery and successfully extubated 1 to 7 days later (Table 4). Two of these patients presented with recurrent severe croup necessitating repeat pediatric intensive care admissions and underwent single-stage LTR as definitive management without ever requiring a tracheotomy. In one patient, mild subcutaneous air collection occurred after the drain was removed, but this minor complication resolved spontaneously.

DISCUSSION

The techniques of surgical correction of LTS have advanced rapidly in recent years. To compare results of surgical therapy, a reproducible classification of LTS is desirable. The Cotton classification[11] divides obstruction into four grades: defined as <70% airway compromise of the cross-sectional area (grade 1), 70% to 90% compromise (grade 2), >90% compromise (grade 3), and complete obliteration of the lumen (grade 4). An alternative system with specific terms and objective parameters has been reported,[12] but it is not widely used. Furthermore, neither system accounts for the more difficult clinical situation of posterior glottic fibrosis, which adds complexity, alters management, and affects prognosis. The classification system proposed here (Table 3) is simple, reproducible, and accounts for the presence of posterior glottic fibrosis. Posterior glottic fibrosis must be distinguished from an interarytenoid web, in which there is a mucosally lined tract posterior to the web, because interarytenoid webs are often successfully

TABLE 4. PATIENTS UNDERGOING SINGLE-STAGE LARYNGOTRACHEAL RECONSTRUCTION

Patient	*Age at Surgery (y)*	*Class*	*Procedure*	*Days With ET*	*ET Size (mm)*	*Comments*
26	2	I	AG	6	4.5	
27	2	I	AG	7	4.0	
28	5	I	AG	7	5.0	Acquired stenosis (premature infant) presented as recurrent croup requiring repeat ICU admissions; never had tracheotomy
29	2	I	AG	5	4.0	
30	5	I	AG	1	5.0	Congenital subglottic stenosis
31	2	I	AG	4	4.5	Congenital subglottic stenosis with recurrent severe croup requiring repeat ICU admissions; never had tracheotomy

ET — endotracheal tube, AG — anterior costal cartilage graft, ICU — intensive care unit.

TABLE 5. RESULTS CORRELATED WITH CLASSIFICATION IN CHILDREN WITH SHORT STENTS PLACED (N = 22)

Class	Patients Decannulated No.	Patients Decannulated %	Average Stent Duration (mo)	Average No. Endoscopic Procedures	Average Time to Decannulation (mo)
I	2/2	100	1.5	2	8
IP	2/3	67	1	3	4
II	8/9	89	2	2.4	5.8
IIP	3/3	100	1	3.7	7
III	3/5	60	12.4	3.7	11.3

treated by endoscopic division.[13] This classification system uses a universal instrument, the 2.7-mm Hopkins 0° telescope, to determine if the lumen is larger than 2.7 mm. This telescope has a flat tip, not the beveled shape of the end of a bronchoscope or endotracheal tube, and therefore the telescope does not dilate the lumen.

A new staging system should be based on consistency of reporting, prognostic ability, and help in treatment decision-making. The simple, reproducible measurement with a standard telescope would allow comparison of data between institutions. The prognosis of LTS is affected by posterior glottic fibrosis. The procedure performed must involve division of the posterior cricoid lamina. The results of decannulation, vocal quality, and swallowing in these patients should be analyzed separately. Also, children with lumens larger than 2.7 mm and without posterior glottic fibrosis (class I) were considered for a single-stage procedure. Those children with smaller airway lumens (class II) most likely need indwelling stents. Those without a functional lumen (class III) will need long-term stenting.

Concerning the use of a short versus long Aboulker stents, the standard long stent with a wired metal tracheotomy tube was associated with a complication in both patients in whom it was placed. The first, a broken stent, was endoscopically removed without sequelae, but the second involved a hypoxic event (in a chronic care facility). Although short Aboulker stents have not been widely used because of the reported risk of suprastomal circumferential granulation tissue, especially when left in place for over 4 weeks,[14] they were found to be relatively safe and effective, and the granulation tissue, which was not circumferential, was successfully managed endoscopically in this series.

Short stents were used for 4 to 6 weeks if an anterior or posterior graft was placed in class I or II patients, but left 2 to 6 months in patients who had posterior cricoidotomies without grafts placed, and up to 23 months in class III patients. Management was as follows. The stent was sharply divided at the appropriate length; then the inferior edge was carefully smoothed with a Bovie cleaner. The stent was secured at the time of LTR with a 2.0-gauge polypropylene suture through the cricoid cartilage and the stent; this suture was tied in multiple knots superficial to the right-side infrahyoid musculature. The stent was then removed endoscopically between 1 and 17 months after placement, after its securing suture was severed via a small cervical incision, but no other manipulation took place at that time. Antibiotics (amoxicillin) and steroids (prednisone, tapered for 1 month) were started. One month later, suspension laryngoscopy was performed. When significant granuloma occurred, it was removed endoscopically. Daily tracheotomy tube changes were done to decrease bacterial colonization, and patients were scheduled for a repeat examination and possible decannulation 1 month after the procedure.

The use of short stents allowed decannulation in 82% (18 of 22) of patients. Because the short stent was not secured to the tracheotomy tube, the possible complication of inability to remove an occluded tube was avoided. The expected suprastomal granuloma (pedunculated, not circumferential) responded to medical and surgical treatment. The average time from stent removal to decannulation in the 18 of 22 children with short stents was 9.2 months; 10 of the 18 (56%) were decannulated within 6 months, and 15 (83%) within 1 year. This is similar to the previously reported average times to decannulation of 9.4 months[2] and 7.0 months.[3] Cotton[13] reported that 36 (61%) of 59 patients were decannulated within 26 weeks, and 49 (83%) of 59 were decannulated within 52 weeks of removal of the stent. In our practice a long stent would be used for the less common stenosis that involves the trachea at or below the level of the tracheotomy stoma. One child had an anterior cartilage graft placed without an intraluminal stent and was not decannulated for 3 years because of slight graft displacement into the lumen (emphasizing the need for stenting) and the need for further treatment of his bronchopulmonary dysplasia.

Posterior glottic fibrosis remains a difficult clinical entity. If a posterior cricoid split without a graft is performed, then we recommend long-term stenting. Placement of a posterior cartilage graft, however, allows a shorter period of stenting and has produced excellent rates of decannulation and satisfactory postoperative voice quality.[13,15] These results should be compared separately from anterior stenoses treated with anterior grafts only, as the anatomically distinct posterior glottis is involved and objective measurement of postoperative vocal quality remains difficult.

Posterior grafts can be difficult to secure and tend to prolapse into the airway. After completion of the posterior cricoidotomy, a small amount of the posterior perichondrium was elevated with a Cottle elevator, and the flange-shape graft was placed (see Figure, inset). This modification provided a more secure fit, allowing for fewer intraluminal sutures.

Problems in the management of three patients who failed decannulation illustrate important points. First, intrinsic cartilage abnormalities may predispose LTR to failure, as in the patient with spondylometaphyseal dysplasia — an observation that has been previously reported.[16] Second, a child with a posterior cricoid split and anterior graft for a class III lesion experienced persistent aspiration postoperatively. Perhaps the superior portion of the stent was too high and was not protected during swallow. The decision was made to plug the hole in the Aboulker stent endoscopically. The plug stopped the aspiration, and the stent was removed 8 months later. One month after removal of the stent, the subglottic space was of normal size and shape, but a new stenosis was discovered in the suprastomal area. This was the only such complication. Whether stent plugging might have led to the new stenoses by allowing secretions to pool remains unanswered. Interestingly, this new stenosis responded to endoscopic carbon dioxide laser division, prompting the question whether the duration of stenosis has implications for treatment. Third, the youngest child (21 months) had an anterior graft for class II stenosis. A 6-mm stent was removed after 1 month. Two weeks later the child's tracheotomy tube was dislodged at home and could not be replaced by parents or paramedics. On arrival to the emergency department, he was asymptomatic, but on endoscopic examination the airway was felt to be marginal for decannulation in the autumn, so the tracheotomy tube was replaced, with decannulation deferred until spring. The patient's small size may have been a factor in outcome, but LTR converted a critical airway to one adequate to sustain accidental decannulation. This child would certainly have not survived a similar accident prior to surgery.

Revision surgery has not been performed in this series. Others have reported a need for revision in 18.5% (5 of 27)[3] to 26% (7 of 27) of patients.[6] Four of 24 (17%) patients, 2 class II and 2 class III, were not decannulated until 2 years after LTR. Revision surgery may have hastened the decannulation process.

In this series, six patients who underwent single-stage LTR were successfully extubated. They were carefully selected for this procedure on the basis of three criteria: a lumen greater than 2.7 mm (class I), normal laryngeal function, and no posterior glottic fibrosis. Two patients with recurrent severe croup had this procedure performed as the primary intervention and never underwent a tracheotomy. The single-stage procedure allows definitive management of LTS and excision of the tracheocutaneous fistula.

In conclusion, the long Aboulker stent is useful in selected patients, but in this series most patients were adequately managed with short stents, avoiding the potential risks of a stent secured to a tracheotomy tube. A careful application of established techniques provides a satisfactory solution for most children with LTS. The proposed system for classifying LTS is based on objective measurement and the presence or absence of posterior glottic fibrosis. The combination of LTS with posterior glottic fibrosis affects the operative procedure and prognosis, thus requiring separate analysis. This classification system also provides guidance for treatment recommendations: class I may be considered for a single-stage reconstruction, class II for short-term stenting, and class III for a longer duration of stent placement.

ACKNOWLEDGMENTS — The authors thank Jonathan Cohen, PhD, and Steffi-Lynn Dahl, Department of Surgery, SUNY–Stony Brook, for their help in preparing the manuscript.

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FIRST CONGRESS OF EUROPEAN SKULL BASE SOCIETY

The European Skull Base Society will hold its first congress September 26-30, 1993, in Riva del Garda (Trento), Italy. For further information contact Carmelo Zappone, MD, Congress Secretary, Division of ORL, Ospedali Riuniti, Largo Barozzi 1, 24100 Bergamo, Italy; telephone 35-269578; fax 35-269460.

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Ann Otol Rhinol Laryngol 102:1993

VOCAL CORD PARALYSIS CAUSED BY ESOPHAGEAL CANCER SURGERY

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Esophageal cancer surgery was the cause of paralysis in 111 (16.7%) of 664 patients with vocal cord paralysis seen from 1981 to 1990 at Kurume University Hospital. Eighty-six patients had unilateral paralysis, whereas 25 had bilateral lesions. Of the 111, 95 (86%) had hoarseness and 59 (53%) had aspiration. The crude survival rate was 63% for 1-year, 32% for 3-year, and 22% for 5-year follow-up. The average life expectancy was short. The general condition was poor in many patients. Thirty-four of the 136 paralytic vocal cords (25%) recovered mobility. An intracordal silicone injection was done in 30 patients, and a medialization procedure was performed in 1. The vocal function was improved in the majority of cases. In 4 patients, severe aspiration persisted following intracordal injection. Of the 4, 1 had cricopharyngeal myotomy and laryngeal suspension and 2 underwent total laryngectomy. The fourth patient died of aspiration pneumonia. The results of the review of the 111 patients have led us to the following treatment policy for patients with vocal cord paralysis caused by esophageal cancer surgery. When the patient has aspiration, vocal cord medialization should be performed as early as possible. If severe aspiration persists even after the medialization procedure, some additional intervention, including cricopharyngeal myotomy, laryngeal suspension, laryngotracheal separation, or total laryngectomy, should be considered. When hoarseness is the only major problem, the prognosis of vocal cord paralysis should be determined on the basis of the state of the recurrent laryngeal nerve and, if available, electromyography findings. Vocal cord medialization is indicated for patients with a poor prognosis for paralysis, and also for those patients whose life expectancy is short.

KEY WORDS — esophageal cancer surgery, intracordal injection, vocal cord paralysis.

During the 10-year period from 1971 to 1980, a total of 564 patients with vocal cord paralysis (VCP) were examined at Kurume University Hospital. Of the 564 patients, VCP was caused by esophageal cancer surgery (ECS) in 24 (4.3%).[1] During the next 10-year period, from 1981 to 1990, 664 patients with VCP were seen at our hospital, and ECS was the cause of VCP in 111 patients (16.7%). The marked increase in patients with VCP caused by ECS resulted from changes in the technique of ECS: a radical dissection of the paratracheal lymph nodes in the neck and upper mediastinum[2,3] was extensively conducted at the Department of Surgery during the latter decade. The surgical technique for the primary lesion was basically the same for the two decades: a thoracic esophagectomy through a right thoracotomy. The esophagus was reconstructed with the use of the stomach. In seeing patients, our attention has been called to some clinically important aspects of VCP caused by ECS. The purposes of this paper were to review the 111 patients, to point out problems associated with VCP caused by ECS, and to propose a treatment policy for this disorder.

SUBJECTS

The subjects were those 111 patients with VCP resulting from ECS who were seen in the Department of Otolaryngology–Head and Neck Surgery, Kurume University Hospital, during the period from 1981 to 1990. Of the 111, 98 were men and 13 were women. Ages ranged from 42 to 81 years, with a mean of 61 years. The lesion was unilateral in 86 cases (right 7, left 79) and bilateral in 25. The ECS had been conducted at the Department of Surgery, Kurume University Hospital, in 106 patients, and 5 patients had undergone ECS in other hospitals.

The clinical charts of the 111 patients were retrospectively reviewed relative to the problems of the patients, outcome of the ECS, prognostic aspects of VCP, and treatments for problems caused by VCP.

RESULTS

Patients' Problems. Of the 111 patients, 95 (86%) had hoarseness. During the same period from 1981 to 1990, 553 patients with VCP from causes other than ECS were seen at our department, and 486 (88%) of the 553 patients had hoarseness. The incidence of hoarseness did not differ significantly between the two groups. The cause of VCP in the latter group was central nerve lesion in 16, thyroid cancer in 32, esophageal cancer in 30, lung cancer in 64, medias-

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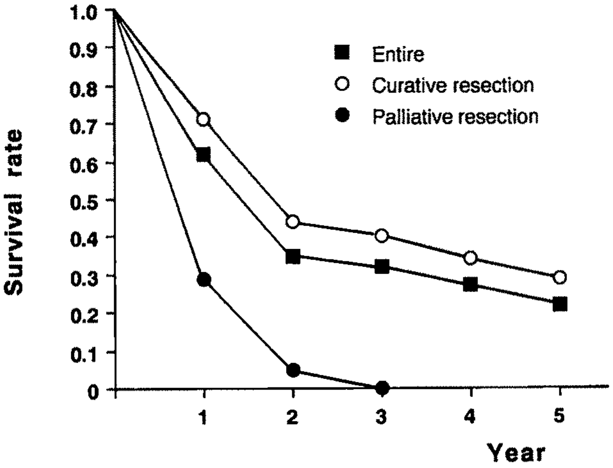

Fig 1. Crude survival rate for 106 patients who were subjected to follow-up study.

tinum tumor in 11, miscellaneous tumor in 4, neck trauma in 5, thyroid surgery in 73, lung surgery in 34, aorta surgery in 20, heart surgery in 10, mediastinum surgery in 9, miscellaneous surgery in 5, endotracheal intubation in 80, pulmonary tuberculosis in 9, aneurysm in 12, heart disease in 6, miscellaneous lesions in 8, and unidentified reasons (presumably neuritis) in 125 patients.

Fifty-nine (53%) of the 111 patients complained of aspiration. The incidence of complaints of aspiration was significantly higher ($p < .01$, χ^2 test) than that for VCP from other causes (64 of 553 or 12%). Those patients who complained of aspiration usually also had a weak cough. No objective evaluation for aspiration was systematically conducted in the present series except that 50 patients (45%) had episodes of aspiration pneumonia. The criteria for the diagnosis of aspiration pneumonia were episodes of aspiration associated with a rise in temperature to 38°C or higher and leukocytosis up to 10,000/μL or more. Twenty-eight patients (25%) had undergone tracheostomy in order to manage persistent aspiration.

Outcome of Esophageal Cancer Surgery. The five patients who had undergone ECS at other hospitals were lost to follow-up. Therefore, the outcome of ECS was determined for the 106 patients who had had surgery at Kurume University Hospital. They were followed up for 1 to 9 years. Of the 106, 82 patients had received curative resection, whereas 24 patients had undergone palliative resection.

Figure 1 depicts the crude survival rate. The survival rate of the entire series was 63% for 1-year, 32% for 3-year, and 22% for 5-year follow-up. Many patients died rather shortly after ECS. All patients with palliative resection died within 3 years following surgery. Even for the patients with curative resection, the 3-year crude survival rate was 40%, and the 5-year survival, 29%.

TABLE 1. PROGNOSIS OF VOCAL CORD PARALYSIS

Recurrent Laryngeal Nerve	*n (sides)*	*Recovered*	*Not Recovered**	*Lost to Follow-up Within 6 Months†*
Sectioned	10	0	3	7
Preserved	60	24	12	24
Unknown	66	10	23	33
Total	136	34	38	64

*At least 6 months of follow-up.

†Patients who died within 6 months are included.

Prognostic Aspects. According to the information from the surgeons who had conducted ECS, the recurrent laryngeal nerve (RLN) had been sectioned on 10 sides and preserved on 60 sides. In the remaining 66 sides they were not sure whether or not the RLN had been preserved. Table 1 shows the prognosis of VCP. A total of 34 of 136 paralytic vocal cords (25%) recovered mobility. Naturally, a recovery of the vocal cord mobility did not take place in the patients whose RLN had been sectioned. When RLN had been preserved during ECS, 24 (40%) of the 60 vocal cords regained mobility. When no information was available relative to injury of the RLN during ECS, 10 (15%) of the 66 vocal cords recovered mobility. The recovery rate was low.

Electromyography (EMG) of the thyroarytenoid muscle was conducted in a total of 47 paralytic vocal cords of 41 patients for prognostic information. For 42 vocal cords, the RLN had been preserved or unidentified during ECS, whereas the RLN had been sectioned on 5 sides. Table 2 shows the relationship between EMG findings and prognosis for the former 42. The latter 5 showed electrical silence or fibrillation potentials in EMG and did not recover vocal cord mobility. As shown in Table 2, the rate of recovery of vocal cord mobility was highest for the Normal group, lowest for the Silence group, and in between for the Reduction group.

In 70 patients, EMG was not performed. We thought these patients would not tolerate EMG because of their poor general condition and/or emotional depression caused by the disease and extensive surgery.

Treatments. For glottic incompetence, an intracordal silicone injection[4,5] was performed in 30 patients and medialization surgery[6] was performed in 1 patient. The intracordal injection was conducted through the cervical skin and cricothyroid space under fiberscopic monitoring. The details of the technique have been reported elsewhere.[5] Of the 31, 17 patients had had aspiration in addition to hoarseness. The voice was subjectively improved in 23 patients and unchanged in 3. No evaluation was available in 5. In

TABLE 2. ELECTROMYOGRAPHIC FINDINGS AND OUTCOME FOR 42 VOCAL CORDS

	Silence or Fibrillation		*Reduction in Active Motor Units*		*Normal*	
Duration of Paralysis	*n*	*Recovery*	*n*	*Recovery*	*n*	*Recovery*
<1 mo	8	2	6	1	1	1
1-3 mo	8	1	13	6	4	3
3-6 mo	0	0	2	1	0	0
Total	16	3	21	8	5	4

1 of the 3 unsuccessful cases, the glottic gap was too big to be corrected by injection. In the second unsuccessful case, the patient was under postoperative radiotherapy for the neck and chest. Her vocal cord mucosa was stiff. The injection yielded a good glottic closure but no voice improvements. In the third case, the patient's voice was improved immediately after injection, but it worsened a month later, when his general condition became worse.

Preoperative and postoperative voice evaluations were conducted in 19 patients who had received intracordal injection. The other 12 patients did not come back for postoperative voice tests. Figure 2 depicts the results. Following injection, the maximum phonation time significantly increased, the mean airflow rate during phonation over comfortable duration decreased, and the sound pressure level range of phonation increased ($p < .01$, paired t test). The fundamental frequency range of phonation did not change significantly.

Aspiration was solved in 13 of the 17 patients, but in 4 patients, intracordal injection was not effective for aspiration. Cricopharyngeal myotomy and laryngeal suspension surgery were conducted in 1 of the 4 patients, and he became able to eat orally. He died of recurrent disease 2 months later. Two patients underwent total laryngectomy for severe aspiration. Both died of recurrent disease at 1 month and 4 months, respectively. The fourth patient did not have any further treatment after intracordal injection because of his poor general condition, and he died of aspiration pneumonia 40 days after the intracordal injection.

COMMENTS

The results of the present study indicate that patients with VCP caused by ECS have the following problems: 1) their average life expectancy is short, 2) many patients are in poor general condition, 3) they often have severe aspiration, and 4) their problems caused by the glottic incompetence are frequently more distressing than the problems caused by VCP in an otherwise healthy population.

It has been advocated that surgical interventions, including intracordal injection, for VCP should be delayed for 6 to 12 months, because spontaneous recovery from paralysis or compensatory overadduction of the unaffected vocal cord takes place during this period.[6-8] This policy of 6 to 12 months of watching and waiting, however, should not be applied for some conditions, including VCP caused by carcinoma and other irrecoverable nerve injuries.[9] The results of the present study indicate that the policy of 6 to 12 months of waiting should not be employed for the majority of patients with VCP caused by ECS. Immediate improvements in the quality of life are frequently demanded.

Our current treatment policy for patients with VCP resulting from ECS is as follows.

1. When the patient has aspiration, vocal cord medialization is performed as early as possible. The first choice of medialization technique is the transcu-

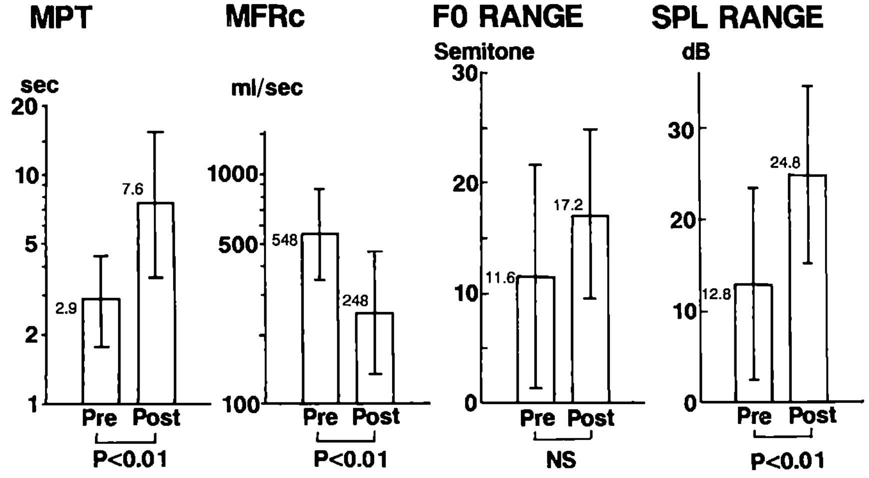

Fig 2. Preoperative and postoperative vocal function for 19 patients. Open rectangle — average value, tailed bar — SD, MPT — maximum phonation time, MFRc — mean airflow rate during phonation over comfortable duration, F0 — fundamental frequency, SPL — sound pressure level.

taneous intracordal injection,[4,5] because it is the least invasive technique currently available.

2. If severe aspiration persists even after intracordal injection, cricopharyngeal myotomy, laryngeal suspension, or total laryngectomy is considered, depending on the patient's condition. A laryngotracheal separation[10-13] appears to be a choice for some selected cases. We have not employed this technique as yet.

3. When the patient has no aspiration and hoarseness is the only major problem caused by VCP, the prognosis for the paralysis is determined on the basis of the state of the RLN (sectioned, preserved, or unidentified) and, if available, the EMG findings. An intracordal injection is conducted when the prognosis of paralysis is unfavorable. An intracordal injection is indicated also in those patients whose life expectancy is estimated to be short.

4. Intracordal injection is occasionally not very effective in patients with a large glottic gap during phonation. Arytenoid adduction surgery[14] may be the choice for such cases.

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4TH INTERNATIONAL ACADEMIC CONFERENCE IMMUNOBIOLOGY IN OTOLOGY, RHINOLOGY AND LARYNGOLOGY

The 4th international academic conference Immunobiology in Otology, Rhinology and Laryngology will be held in Oita, Japan, April 4-7, 1994. For further information contact Goro Mogi, MD, Dept of Otolaryngology, Oita Medical University, Hasama-machi, Oita 879-55, Japan; telephone 81-975-49-4411 ext 2912 or 2913; fax 81-975-49-0762.

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CONGENITAL ORAL SYNECHIAE

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Major congenital synechiae of the oral cavity constitute a clinically confusing spectrum of abnormalities. On the basis of clinical data, we propose two categories: 1) abnormalities secondary to persistence of the buccopharyngeal membrane and 2) abnormalities secondary to formation of ectopic membranes. An ectopic membrane results from abnormal fusion and can be subclassified as a subglossopalatal membrane, glossopalatal ankylosis, or syngnathia. This classification is supported by embryologic studies and is used to reclassify all cases reported since 1900. Distinct differences, such as the presence of associated limb anomalies, emerge; these are reviewed and add support to the proposed classification.

KEY WORDS — adhesions, ankylosis, congenital defects, ectopic tissue, jaw abnormalities, mouth abnormalities.

INTRODUCTION

Congenital adhesions (synechiae) between different parts of the oral cavity rarely occur. While these synechiae most commonly arise between the upper and lower alveolar ridges (syngnathism) or between the tongue and margins of the palate or maxilla (glossopalatal ankylosis), synechiae arising from the lower lip, the floor of the mouth, or at the oropharyngeal isthmus have been described. They may consist of membranes or bands of epithelium supported by various amounts of connective tissue, and possibly even muscle or bone. Almost invariably, they are accompanied by one or more additional congenital defects such as cleft palate (CP), cleft lip, microglossia, micrognathia, temporomandibular joint (TMJ) ankylosis, or limb anomalies. In several cases, a familial occurrence has been demonstrated.

Many causes for the development of intraoral synechiae have been proposed, with persistence of the buccopharyngeal membrane (BPM) being most commonly cited, although good supportive embryologic evidence is lacking. In this report of two cases, we have included our interpretation of the origin of aberrant oral adhesions, based on the embryology of the oral cavity and a review of the literature since 1990.

CASE REPORTS

Case 1. A 2,650-g male infant was delivered vagi-

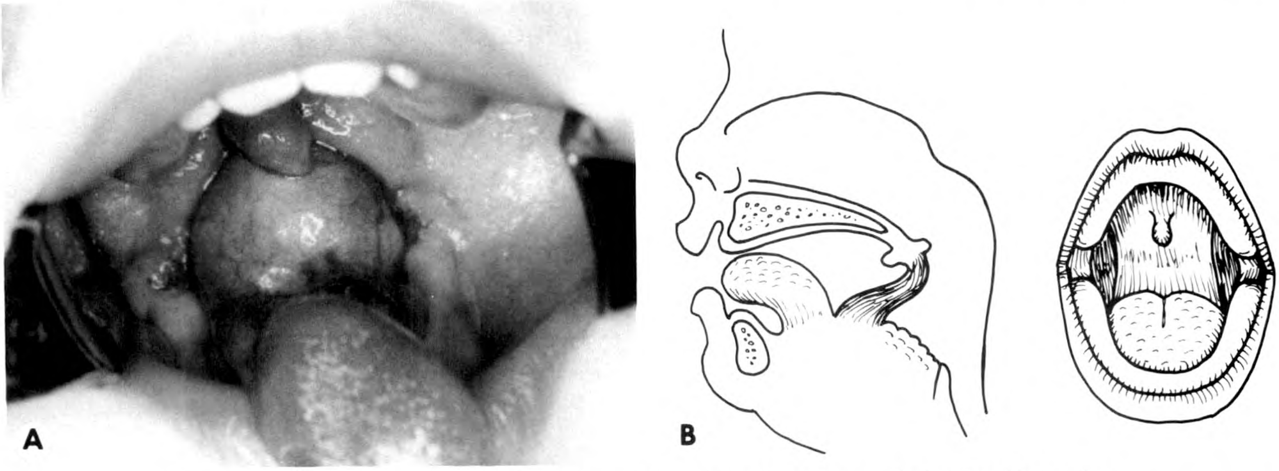

Fig 1. (Case 1) Persistent buccopharyngeal membrane. A) Anterior intraoral view. B) Schematics.

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†Deceased.

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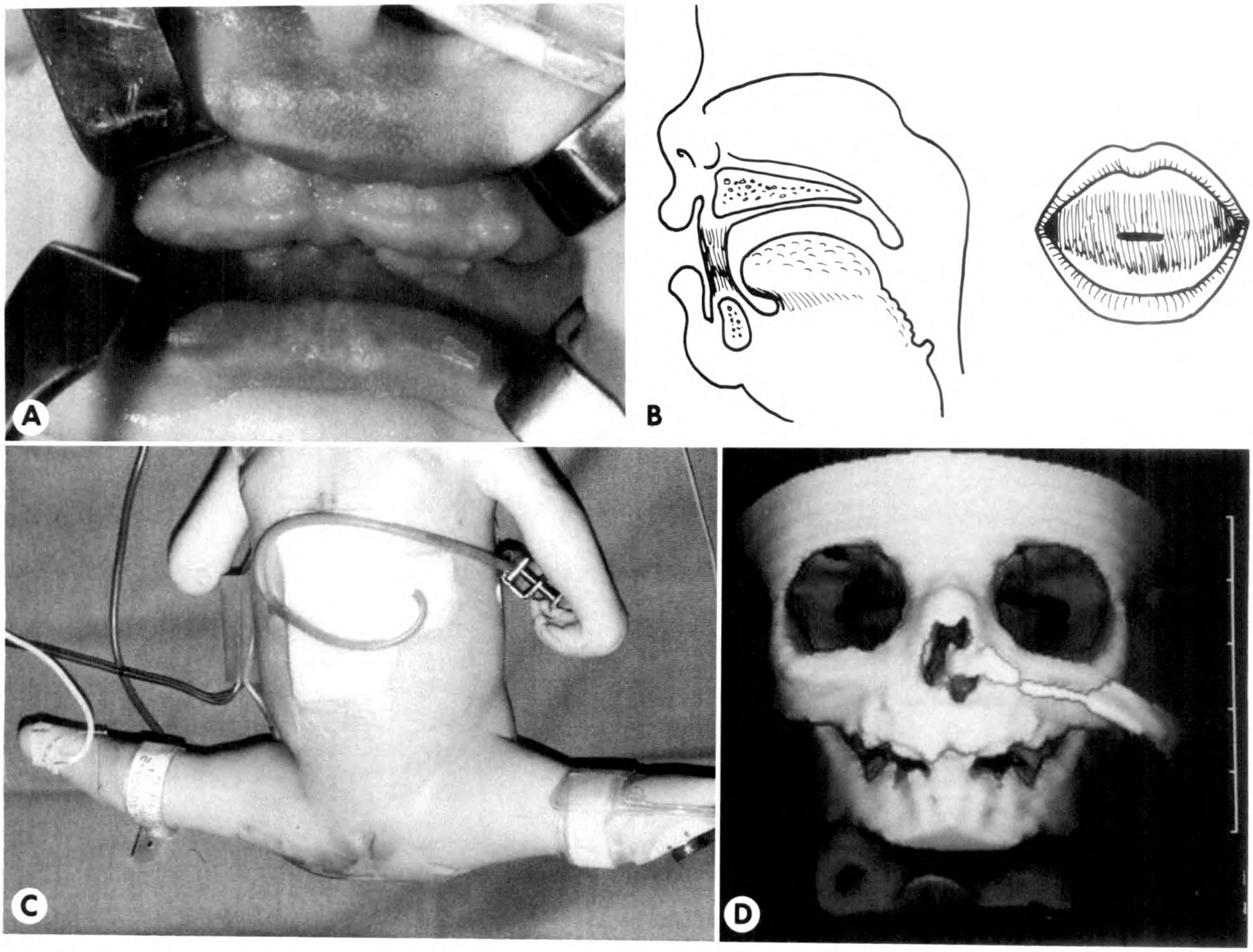

Fig 2. (Case 2) Syngnathism. A) Anterior intraoral view, demonstrating fibrous and bony fusion of alveolar ridges. B) Schematics. C) Multiple limb anomalies. D) Interalveolar bony fusion.

nally following a term pregnancy complicated by polyhydramnios. The mother denied medication use during pregnancy. The Apgar scores were 6 and 8 at 1 and 5 minutes, respectively. Soon after birth, the child developed respiratory distress that did not improve with masked ventilation. Repeated intubation attempts failed, and the neonate was transferred to The University of Iowa Hospitals and Clinics, where an emergency tracheotomy was performed. Endoscopic examination was not possible because of previous intubation trauma; however, a high arched palate, an incompletely formed tongue, microglossia, micrognathia, a large uvula, and a tear in the posterior oropharyngeal wall were noted. Postoperative feeding difficulties prompted a videoesophagram, which demonstrated absent deglutition and minimal tongue mobility. The patient was discharged on nasogastric feedings.

One year later, the infant was referred to the pediatric otolaryngology service for sialorrhea, feeding difficulties, and absent oral reflexes. Examination under anesthesia revealed an apparently complete membranous septum extending from the posterior surface of the soft palate down to the base of the tongue along the anterior faucial pillar (Fig 1). The anterior and posterior aspects of the tongue were divided by this membrane, and a pinpoint communication was identified between the oral cavity and the pharynx to the right of the midline. Surgical correction was performed by excision of the oropharyngeal partition with glossoplasty. No tonsillar tissue was found, and two distinct uvulae were identified, one lobe extending orally and the other posteriorly into the pharynx. The nasopharynx and larynx appeared normal.

Case 2. A 2,260-g, 38-weeks-gestation female infant was delivered by cesarean section necessitated by breech presentation. Resuscitation with oxygen and chest compression for 1 minute were immediately required; her 1- and 5-minute Apgar scores were 5 and 8, respectively. Examination demonstrated syngnathism (Fig 2A,B), and a tracheotomy was done to relieve persistent respiratory difficulty. Pertinent physical findings included bilateral leg and right arm amputations, fused knees, abducted and hyperflexed hips, and a malformed left lower arm

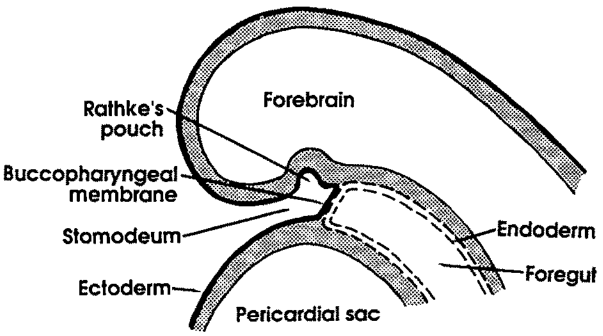

Fig 3. Schematic of third week of embryogenesis.

and hand (Fig 2C). The ophthalmologic, urologic, and audiologic examinations showed normal findings, as did an electrocardiogram and a chromosome analysis. However, the electroencephalogram was abnormal, and decreased myelinization of the pons was demonstrable by magnetic resonance imaging. Computed tomography with three-dimensional reconstructions demonstrated mandibular hypoplasia with interalveolar bony fusion in the midline (Fig 2D). The TMJs appeared radiographically normal. There was no family history of congenital anomalies, nor a history of drug use during pregnancy. Gastrostomy and fundoplication were performed, and the syngnathism was released by dividing the fibrous interalveolar fusions and the 1.0-cm bony bridge that connected the right paramedian lower alveolar ridge to the maxilla. Anteriorly, the tongue remnant was completely adherent to the hard palate; this attachment was easily released with minimal bleeding. The distinct superficial anatomy of the tongue was absent. A postalveolar cleft palate extended through the soft palate and the secondary hard palate. Limited mandibular excursion was consistent with fibrous ankylosis of the TMJs. Postoperatively, mandibular movement was maintained by means of manual exercise and a pacifier.

The patient's hospital course was complicated by pneumonia, a seizure disorder, and small bowel necrosis that required surgical resection. After 5 months, the infant was discharged. She later suffered a respiratory arrest, and although resuscitation was per-

TABLE 1. CHRONOLOGY OF HUMAN EMBRYOGENESIS

3rd week (15-21 days)	Reversal of embryo head and tail folds occurs (days 18-19)
	Stomodeum deepens
	Buccopharyngeal membrane slopes upward and backward
	Rathke's pouch develops
	Olfactory placodes appear in frontonasal process
4th week (22-28 days)	Buccopharyngeal membrane ruptures and dissolves (day 24)
	Tongue primordia develops (tuberculum impar)
	Olfactory placodes become nasal pits
5th week (29-35 days)	Bilateral lingual swellings form anterolateral to tuberculum impar (days 28-29)
	Premaxillary portion of palate forms (primordial palate; days 33-37)
	Palatal shelves appear (day 35)
	Nasal pit connects with mouth cavity
	Septal process appears
	Limb buds first appear (day 32)
	Rathke's pouch disappears (day 35)
6th week (36-42 days)	Individual components of tongue are in process of fusion
	Marked progress occurs in development of upper jaw
	Vestibule and alveolar ridge begin to develop
	Bucconasal membranes rupture, forming primitive choanae
	Mandibular bone begins to develop
	Upper limbs subdivide into arm, forearm, and hand (day 37)
	Hind limbs are still paddle-shape
	Hand condenses into digital rays (days 40-42)
7th week (43-49 days)	Upper jaw continues to develop
	Palatal shelves are downwardly displaced and separated by tongue
	Tongue is elevated from skeletal muscle differentiation
	Meckel's cartilage (mandibular prominences) fuses in midline
	Definite nasal septum grows
	Digital rays develop in feet
8th week (50-56 days)	Palatal shelves are still separated by tongue, although less downwardly displaced
9th week (57-63 days)	Palatine processes elevate and fuse in midline anteriorly
	Tongue rapidly retracts inferiorly
10th week (64-70 days)	Upper jaw undergoes marked growth relative to mandible (10th-12th weeks)
	Condylar tip cartilage of mandible develops
	Lips separate from gums, forming vestibules
12th week	Palatal fusion is complete
	Choanae become fully established
	Mandibular ramus and condylar cartilage fuse
16th week	Temporomandibular joint acquires definite form
18th week	Coronoid process develops intramembranously and fuses with ramus of mandible

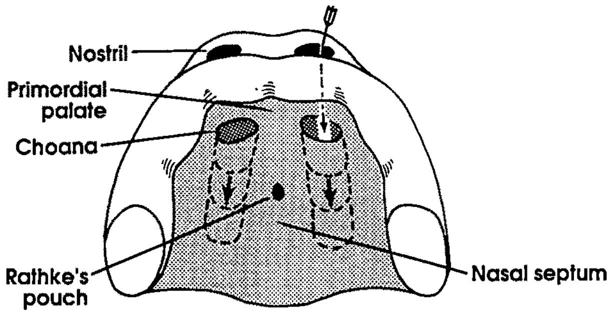

Fig 4. Schematic showing posterior extension of nasal pits and simultaneous lengthening of nasal septum. Rathke's pouch is carried on free edge of nasal septum. (Modified from Davies.[2])

formed by the parents, she died.

EMBRYOLOGY

Origin of Buccopharyngeal Membrane. The BPM is derived from the cranial aspect of two linear areas in the axially differentiated embryonic disc (18 to 19 days) in which the ectoderm and endoderm remain in contact and are never invaded by mesoderm. At this stage, the cranial area of ectodermal-endodermal contact is known as the prochordal plate. It lies immediately anterior to the primitive streak and, at a slightly later stage, anterior to the head (notochord) process, a forward extension of the primitive streak. The caudal area of ectodermal-endodermal contact is the cloacal membrane and does not concern us here.

Rapid craniocaudal growth of the embryonic disc, especially dorsally, results in the so-called "reversal" of the embryo as the disc contracts around the narrowing isthmus of the yolk sac (vitellointestinal isthmus). In this process, the septum transversum and the pericardial sac become displaced caudally beneath the overhanging prominence of the forebrain. The BPM, likewise, is carried beneath the bulging forebrain. Thus, the stomodeum is brought into existence as a transverse slit between the forebrain and the pericardium (Fig 3).

TABLE 2. POSTERIOR BUCCOPHARYNGEAL MEMBRANE REMNANTS

Authors	*Year*	*Intraoral Synechiae*	*Cleft Palate*	*Cleft Lip*	*Micrognathia*	*Microglossia*	*Anomalous Extremity*	*TMJ Ankylosis*	*Other*
Fridenberg[3]	1908	Bilateral supernumerary anterior tonsillar pillars from soft palate to posterior tongue							
Longacre[4]	1951	Inferior extension of velum to base of tongue in region of sulcus terminalus; no tonsil or tongue tissue evident	X						
Seghers[5]	1966	Inferior extension of velum along anterior pillars to base of tongue in region of sulcus terminalis; uvula anterior to membrane							Costovertebral anomaly, auricular malformation
Chandra et al[6]	1974	Inferior extension of velum to base of tongue in region of sulcus terminalis; uvula not seen							
Hoffman[7]	1979	Inferior extension of velum along anterior pillars to base of tongue; uvula not seen							
Hirabayashi and Ueda[8]	1985	Teratoma extending from sphenoid to floor of mouth and inferior alveolus	X						C4-T3 bony spinal defect, intestinal obstruction, normal chromosomes
Arcand and Haikal[9]	1988	Fibrous band from base of tongue to vomer posterior to velum							
Gartlan et al	1992	Inferior extension of velum along anterior pillars to divide tongue in region of sulcus terminalis; anterior and posterior uvulae seen			X	X			Low-set ears, downslanting palpebral fissures, high arched palate

TMJ — temporomandibular joint.

TABLE 3. ANTERIOR BUCCOPHARYNGEAL OR SUBGLOSSOPALATAL MEMBRANES

Authors	*Year*	*Intraoral Synechiae*	*Cleft Palate*	*Cleft Lip*	*Micro-gnathia*	*Micro-glossia*	*Anomalous Extremity*	*TMJ Ankylosis*	*Other*
Lamothe[10]	1930	Gingival adhesions and fibrous bands from hard palate to floor of mouth (tongue absent)					X	X	
Keith[11]	1940	Fibrous band from cleft palate to frenulum	X						Anencephaly
Farrington[12]	1947	Fibrous band from hard palate to floor of mouth			X	X			Prominent sublingual ridges, abnormal teeth, no vestige of tongue
Kouyoumdjian and McDonald[13]	1951	Fibrous band from soft palate to floor of mouth					X		Tracheoesophageal fistula, esophageal atresia, ureteral obstruction, patent foramen ovale, bilateral cataracts, Meckel's diverticulum, adrenal neuroblastoma
Hayward and Avery[14]	1957	Fibrous band from cleft palate to frenulum	X						Family history of cleft palate
Berendes[15]	1960-1961	Fibrous band from cleft palate to floor of mouth	X		X	X			Patient's mother had similar anomalies
Hub and Jirasek[16]	1961	Fibrous band from hard palate to frenulum	X						
Fuhrmann et al[17]	1972	Multiple fibrous bands from hard palate to floor of mouth and lateral tongue	X		X	X			Short upper lip; five members of family had cleft lip with synechia
Nakajima et al[18]	1979	Fibrous band from hard palate to floor of mouth	X				X		Bifid tongue dorsum, patent ductus arteriosus, hydrocephalus
Zalzal et al[19]	1986	Fibrous band from hard palate and retromolar trigones to floor of mouth and anterior tongue	X				X		Hypospadias, bifid scrotum, umbilical hernia
Legius et al[20]	1988	Intrabuccal band from nasal septum to sublingual plicae	X				X		Potter sequence, cardiopathy, bilateral simian creases, normal chromosomes

TMJ — temporomandibular joint.

Soon after this, in embryos 2 to 3 mm in crown-rump length (13 to 20 paired somites; about 24 days), the BPM breaks down and normally disappears without a trace. At its greatest development it cannot be more than a few microns in thickness and in area. It is an ephemeral structure that does not survive the events associated with the rapid differentiation of the pharynx, nose, face, and tongue.

Salient Features of Pharyngeal, Olfactory, and Lingual Development. These events are summarized chronologically in Table 1 and will be described here only insofar as they are of importance in the identification of remnants of the BPM in the newborn or infant. As described above, the BPM is an ephemeral and delicate structure of ectoderm and endoderm, lacking in intervening mesoderm. Its disintegration as a result of tensions occasioned by rapid growth and differentiation of the oropharyngeal structures, as suggested by Watanabe et al,[1] is likely. If it fails to break down, its theoretic sites of attachment can be

TABLE 4. GLOSSOPALATAL ANKYLOSIS

Authors	*Year*	*Intraoral Synechiae*	*Cleft Palate*	*Cleft Lip*	*Micrognathia*	*Microglossia*	*Anomalous Extremity*	*TMJ Ankylosis*	*Other*
Kramer[21]	1911	Ankylosis of tongue to hard palate	X				X		Facial paralysis
Phelip[22]	1920	Ankylosis of tongue to hard palate	X			X	X		Right facial paralysis
Esau[23]	1921	Ankylosis of tongue to upper alveolus							
Jurgenssen[24]	1947	Ankylosis of tongue to nasal septum through cleft	X		X	X	X		Anencephaly, right facial and abducens paralysis
Cosack[25]	1952-1953	Ankylosis of tongue to hard palate			X		X	X	Left facial paralysis, abnormal teeth
Bunnige[26]	1960	Ankylosis of tongue to hard palate			X	X	X		Cleft tongue tip, tongue hypomobility, hypodontia
Wilson et al[27]	1963	Ankylosis of tongue to hard palate				X	X		High arched palate, polyhydramnios
Spivak and Bennett[28]	1968	Ankylosis of tongue to anterior maxilla			X	X	X		Bilateral facial paralysis, left eye antimongoloid slant

TMJ — temporomandibular joint.

predicted from subsequent developmental events, as outlined chronologically in Table 1.

A superior landmark for the site of BPM attachment is Rathke's pouch, which disappears in embryos of about 10 to 15 mm in length (about 35 days). Persistence of any remnant of this pouch (as a pituitary rest in the roof of the nasopharynx) would mark the superior attachment of the BPM, but in general this persistence is too rare to be of practical value.

The upper attachment also lies near the cranial end of the notochord — the site of a craniopharyngioma. However, formation of the olfactory pits and the rapid increase in the anterior-posterior and lateral extent of the roof of the stomodeum make definite identification of the sites of attachment to the base of the skull difficult. The mesodermal bed ventral to the forebrain grows to accommodate the deepening caudal excavation of the nasal pits. The nasal pits eventually open posteriorly into the roof of the stomodeum and form the posterior choanal apertures when the transitory bucconasal membrane breaks down (sixth week of embryogenesis). The area between the pits becomes the primitive nasal septum, and that anterior to the pits becomes the primordial palate (Fig 4[2]). The area of Rathke's pouch can be displaced a considerable distance inferiorly, even onto the posterior surface of the nasal septum, and therefore this displacement would also be reflected in the position of a BPM remnant on the septum or even the soft palate.

Developmental events that affect the lateral attachment of the BPM are associated with those resulting in the formation of the eustachian tube, tonsil, and faucial pillars. The eustachian tube arises from a coalescence of the first and second pharyngeal (branchial) pouches; they are endodermal in origin and accordingly must lie posterior to the line of the BPM. The palatine tonsil arises as a clustering of mesodermally derived lymphocytes around the sequestered ventral wing of the second pharyngeal pouch. The anterior faucial pillar (palatoglossal fold) may represent the line of the first pharyngeal arch, and the posterior faucial pillar (palatopharyngeal fold), the line of the second. Any remnant of the BPM must therefore lie anterior to or along the anterior pillar.

In the floor of the mouth and oropharynx, events are dominated by the development of the tongue. It is represented in embryos of about 5 mm (days 28 and 29) by bilateral lingual swellings in the ventral ends of the first arch, where they meet in the floor of the pharynx immediately posterior to the inferior attachment of the BPM. Together with an unpaired posterior swelling, the tuberculum impar, these structures increase in size and form the anterior two thirds of the tongue, supplied by the mandibular nerve (lingual branch). The posterior third of the tongue is derived from the ventral ends of the third pharyngeal arch and the anterior part of the hypobranchial eminence in the midline and is innervated by the glossopharyngeal

TABLE 5. SYNGNATHISM (NONHEREDITARY)

Authors	*Year*	*Intraoral Synechiae*	*Cleft Palate*	*Cleft Lip*	*Micro-gnathia*	*Micro-glossia*	*Anomalous Extremity*	*TMJ Ankylosis*	*Other*
Kettner[29]	1907	Bony gingival adhesions	X			X	X		Prominent sublingual ridges
Davis[30]	1935	Bony gingival adhesions						X	
Burket[31]	1936	Fibrous and bony gingival adhesions			X				Left Horner's syndrome, left hemifacial atrophy, left mandibular aplasia, port wine stains, polyhydramnios
Szelely[32]	1941	Fibrous gingival adhesions							
Hochstetter[33]	1948	Fibrous gingival adhesions							Noted in skull recovered from Austrian ossuary
Mathis[34]	1962	Fibrous gingival adhesion laterally	X						
Salleh[35]	1965	Bony gingival adhesions			X				
Pettersson[36]	1961	Complete bony gingival adhesions	X		X	X	X		Absent alveolar ridges
Snijman and Prinsloo[37]	1966	Bony gingival adhesions laterally			X			X	No gingivobuccal sulcus
Hoggins[38]	1969	Fibrous gingival adhesions			X	X	X		
Fish[39]	1972	Fibrous gingival adhesions laterally	X						
Miskinyar[40]	1979	Fibrous gingival adhesions			X				
Sarikayalar et al[41]	1982	Fibrous gingival adhesions laterally						X	Right hemifacial atrophy, hypoplastic right mandibular ramus
Randall[42]	1984	Fibrous gingival adhesions							
Narasimharao et al[43]	1984	Fibrous gingival adhesions laterally			X			X	Low-set ears
Seraj et al[44]	1984	Fibrous gingival adhesions laterally	X						
Verdi and O'Neal[45]	1984	Fibrous gingival adhesions	X						
Goodacre and Wallace[46]	1990	Fibrous gingival adhesions laterally	X						
		Bony gingival adhesions laterally							Multiple other congenital anomalies not reported
		Fibrous gingival adhesions laterally	X						
		Fibrous gingival adhesions laterally			X				TMJ dislocation with elongated condyle and exophthalmus
		Fibrous gingival adhesions laterally			X				
Pillai et al[47]	1990	Fibrous gingival adhesions laterally	X						
Gartlan et al	1992	Fibrous and bony gingival adhesions laterally	X		X		X	X	Abnormal pontine myelinization

TMJ — temporomandibular joint.

TABLE 6. SYNGNATHISM (HEREDITARY)

Author	*Year*	*Intraoral Synechiae*	*Cleft Palate*	*Cleft Lip*	*Micrognathia*	*Microglossia*	*Anomalous Extremity*	*TMJ Ankylosis*	*Other*
Neuman and Shulman[48]	1961	Fibrous gingival adhesions	X						Ankyloblepharon, lower lip sinus, genetic predisposition
Fish[39]	1972	Fibrous gingival adhesions laterally	X	X					Mother and maternal grandmother had complete unilateral cleft lip and palate
Shaw and Simpson[49]	1980	Fibrous gingival adhesions laterally	X	X					Bilateral fistulae of lower lip
		Fibrous gingival adhesions laterally	X	X					Bilateral lower lip fistulae; mother had cleft lip
		Fibrous gingival adhesions laterally	X	X					Unilateral lower lip fistula
Sternberg et al[50]	1983	Complete fibrous gingival fusion	X	X	X				Sibling with similar disorder (consanguineous marriage)
Traaholt[51]	1990	Fibrous and bony gingival adhesions laterally	X	X					Ankyloblepharon, downslanting eyes; brother had gingival adhesions

nerve. (The second arch drops out of the floor of the pharynx in this area.)

Rapid elevation of the lingual mass in the floor of the oropharynx results in the formation of a paralingual sulcus from which the submandibular and sublingual glands develop, as a series of outgrowths in the case of the former and by closure of a groove in the case of the latter. These glands are usually considered to be ectodermal (ie, belonging properly to the stomodeum) and so lie anterior to the lower attachment of the BPM. The teeth arise in the alveolar ridges external to the paralingual sulcus and are ectodermal except for their mesodermal pulp.

The tongue, perhaps as a result of the normal hypoplasia of the mandible in these early stages, presses on the inferior surface of the primitive nasal septum posterior to the primordial palate and prevents the maxillary shelves from fusing with the septum. Fusion does not begin until after the tongue withdraws at about the ninth week. If the inferior aspect of the BPM was to persist, it would have to survive the development of the tongue impinging posteriorly on its lower and posterior surface. This survival is unlikely.

Theoretic Attachments of Buccopharyngeal Membrane in Neonates. These have been described by Davies[2] (Fig 5[2]) on the basis of known facts of human embryology. The upper attachment should lie in the roof of the nasopharynx in the region of the basisphenoid, and the pharyngeal tonsil posterior to the site of Rathke's pouch. As explained above, Rathke's pouch can be displaced onto the posterior surface of the nasal septum as development proceeds, so the upper BPM attachments also might be conceivably found low on the septum, close to the attachment of the uvula. More inferiorly and on either side, the attachment of the BPM should lie along a line anterior to the orifice of the eustachian tube and follow the anterior faucial pillars. Further inferiorly and in the midline, the BPM should skirt the tongue along the paralingual sulcus and internal to the alveolar ridge. Of these landmarks, the eustachian tube and faucial pillars are probably the best markers for the position of the BPM.

DISCUSSION

In our review of the world literature since 1900, we have identified nearly 50 reports of anomalous bands of intraoral tissue (Tables 2-6[3-51]). For lack of a better name and for lack of a better understanding of embryogenesis, in most instances this anomaly has been attributed to persistence of the BPM. This has caused significant confusion and makes attempts to classify this compendium of syndromes and anomalies diffi-

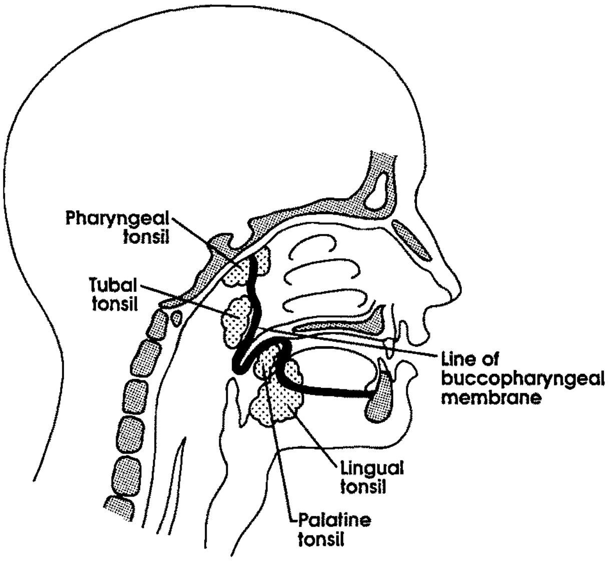

Fig 5. Schematic showing approximate position of former buccopharyngeal membrane (junction of ectoderm anteriorly and endoderm posteriorly). Lymphoid tissue at oropharyngeal isthmus is also shown. (Modified from Davies.[2])

cult.

What is evident from the reported cases is that anomalous bands of intraoral tissue vary in severity and can occur with associated deformities. Most certainly, etiologic differences exist as well. As far as we can tell, complete persistence of the BPM has not been observed. By complete, we mean an intact membrane extending from the roof of the nasopharynx to the anterior faucial pillars and over the tongue to the lingual aspect of the mandible. Such a membrane would obstruct both the oral and nasal passages and be incompatible with life. In 19 cases (Tables 2 and 3), however, an embryologic remnant of the BPM is described (8 posterior, 11 anterior). Several additional cases have been reported to be BPM remnants, but the anomalies were insufficiently described and therefore are not included in this report.[52-54]

Seven of the eight cases (Fridenberg,[3] Longacre,[4] Seghers,[5] Chandra et al,[6] Hoffman,[7] Arcand and Haikal,[9] and our case 1) of posterior BPM remnants involve a membrane that attaches to the base of the tongue and extends superiorly along the anterior faucial pillars to fuse with the soft palate or vomer (Table 2 and Fig 1B). This accords well with the embryologic facts. In four of these cases, the membrane completely separated the oropharynx from the oral cavity; in the other three, it was deficient, suggesting partial rupture. There was no extension across the choanae to the roof of the nasopharynx. Most commonly (in five cases), the BPM attached to the free margin of the soft palate or to its posterior surface, as expected by the mechanism of palatal fusion in embryogenesis. Two cases had an accompanying cleft of the soft palate, perhaps because fusion was prevented by the BPM itself. No case was associated with anomalous extremity deformities.

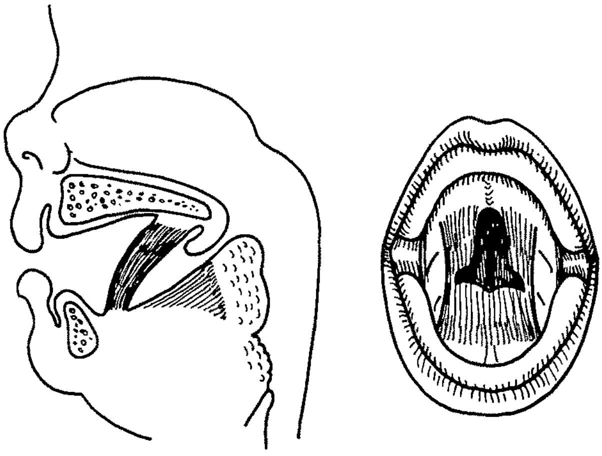

Fig 6. Schematics of subglossopalatal membrane or anterior buccopharyngeal membrane.

Persistent posterior BPMs may be due to mesenchymal tissue entrapment or migration through the endoderm-ectoderm interface. This view is supported by the case of Hirabayashi and Ueda[8]: a neonate had a large teratoma arising from a wide bony connection in the sphenoid and tracking inferiorly through a cleft hard and soft palate to the floor of the mouth, inferior alveolus, and lower lip. Its path traces the embryologic position of the BPM.

The 11 cases of anterior BPM remnants (Table 3 and Fig 6) also have been described as subglossopalatal membranes, reflecting unclear embryologic origins. All of these cases have features suggesting a persistent BPM, and as in some cases in the previous group, the anterior-inferior membranous attachment is the anterior and lateral floor of the mouth. The intraoral bands, however, extend to the palate (hard and soft) and, in cases of clefts, to the margin of the CP or nasal septum. Furthermore, a significant number have been associated with CP (73%), microglossia (36%), micrognathia (27%), extremity deformities (45%), and other congenital anomalies (45%); in 2 cases (Berendes[15] and Fuhrmann et al[17]) there appears to be a genetic predisposition. The high incidence of CP may be due to midline BPM persistence: if a BPM disintegrates only laterally and remains intact medially, palatal closure will be inhibited. In some instances, the BPM then fuses to the margins of the cleft.

However, because of the ephemeral nature of the BPM, especially inferiorly where tongue growth is quite likely to destroy it, classifying this group of anomalies as anterior BPM remnants may be suspect, and an alternative explanation is possible. Zalzal et al[19] and Nakajima et al[18] have postulated that the membranes in these cases arise during the seventh week of embryogenesis, when the enlarged tongue

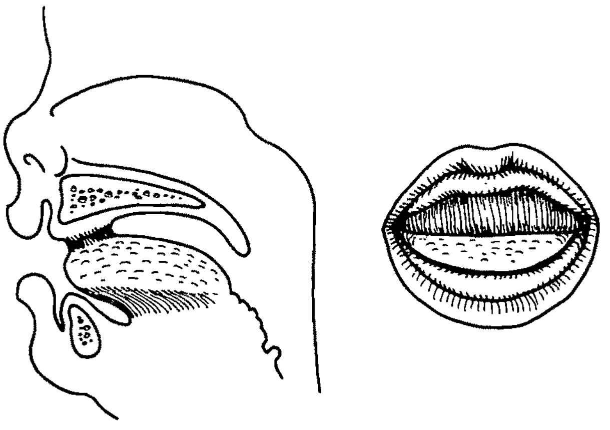

Fig 7. Schematics of glossopalatal ankylosis.

causes the palatal shelves to project into the floor of the mouth. The epithelium of the palatal shelves and the floor of the mouth contact each other and fuse. When the vertical dimension of the face increases with continued normal growth, the restriction imposed by these tissue bands elevates the floor of the mouth and deviates the tongue posteriorly. The result is a subglossopalatal membrane, a membrane that attaches inferiorly to the floor of the mouth and superiorly to the margin of the cleft or soft palate.

Definitive classification of any of these 11 cases (Table 3) as either an anterior BPM or a subglossopalatal membrane (SGPM) is difficult and probably impossible. However, if one is to attribute any of these abnormalities to abnormal fusion, it is relevant to consider the fusion capabilities of embryonic oral tissue in general. Goss et al[55] studied rats during an embryonic period analogous to the seventh week in humans and concluded that fusion is not limited to specific areas on the palatal shelves. Rather, it is a general property of all embryonic oral tissues at this stage. However, for fusion to occur, tissues must be in close and quiescent contact. For this reason, tongue-palate fusions are rare, since the necessary neuroanatomic pathways and muscular differentiation for orofacial movement are present prior to this time and movement is therefore present.

Further supporting the SGPM theory of abnormal fusion and weakening the anterior BPM theory is the presence of other anomalies that have not been seen in the posterior BPM group. These additional anomalies suggest a more global environmental or genetic insult during the sixth or seventh week of development. This hypothesis can be supported experimentally. For example, Bodner et al[56] have demonstrated glossopalatal fusion in rats treated with the tranquilizer chlorcyclizine, perhaps secondary to inhibition of movement or to the teratogenic effect of chlorcyclizine itself. Drugs such as meclizine have also been shown to cause glossopalatal fusion, CP, micrognathia, and micromelia by upsetting skeletal growth and causing edema.[57] Warkany et al[58] were able to induce CP, micrognathia, and malformation of the extremities in rats by feeding mothers a nutritionally deficient diet.

The dynamics of palatal closure must also be considered. Intuitively it seems that space is available above the tongue for the horizontal positioning of the palatal shelves; however, Wragg et al[59] and Greene and Kochhar[60] have shown that this space is an artifact. The tongue grows at the same rate as the lower jaw and is in close contact with the palate and both alveolar ridges. It is contraction of the tongue and other orofacial muscles that leads to a dorsal lingual depression that facilitates a rotatory movement of the palatal processes.[59] These semigelatinous structures shift in a hingelike fashion to a horizontal position, and the tongue then occupies the area vacated by the palatal processes. Therefore, in addition to environmental or teratogenic factors, failure of fusion of the palatal shelves also may occur if the tongue does not descend. Walker[61] has shown that experimental amniocentesis in mice compresses the fetus and causes CP by reducing the pressure in the amniotic sac. This manipulation results in an abnormally flexed head position with the head pushed into the chest, secondarily forcing the mandible and tongue upward.

The remaining three groups of cases, detailed in Tables 4-6, involve anteriorly occurring synechiae of the alveoli or palate. These synechiae have been classified as glossopalatal ankylosis, syngnathism, ankyloglossium superius, aglossia-adactylia syndrome, congenital TMJ ankylosis, congenital fusion of the gums, congenital alveolar synechiae syndrome, congenital aglossia, and persistent BPM. Our case 2 is illustrative and demonstrates several associated anomalies, including glossopalatal ankylosis, syngnathism, and extremity deformities.

In general, we believe that all synechiae in the anterior oral cavity can be adequately described by either syngnathism or glossopalatal ankylosis. Syngnathism implies congenital adhesions of the jaws, ranging in severity from membranous adhesions to complete bony fusion of the alveolar ridges (Fig 2). Glossopalatal ankylosis, in contrast, describes congenital fibrous or bony adhesions between the tongue and the palate or maxilla (Fig 7). Like SGPM, syngnathism and glossopalatal ankylosis can be caused by either genetic or environmental influences, but irrespective of the cause, the mechanism of formation appears to be the same.

During the seventh to eighth week of development, the alveolar ridges, tongue, and palatal shelves are in contact with each other. As mentioned previously, the ensuing palatal closure depends on downward contraction of the tongue.[59] As the tongue protrudes from the mouth as a result of medial movement of the oral cavity walls, it prevents fusion of the alveolar ridges. Genetic, teratogenic, or mechanical insults during this critical stage may lead to periods of close, quiescent contact between oral structures and predispose to the development of abnormal fusions. A constellation of anomalies associated with inadequate neuromuscular development of orofacial, tongue, palatal, and mandibular structures, including hemifacial atrophy (Burket[31] and Sarikayalar et al[41]), facial paralysis (Kramer,[21] Phelip,[22] Jurgenssen,[24] Cosack,[25] and Spivack and Bennett[28]), tongue hypomobility (Bunnige[26]), microglossia, aglossia, CP, and micrognathia, support this hypothesis. Of nonhereditary cases of syngnathism (Table 5), 67% involved fibrous synechiae, with at least some bony component in 38%. Other anomalies included CP (42%), micrognathia (42%), microglossia (13%), TMJ ankylosis (25%), and limb deformity (17%). In comparison, cases with glossopalatal ankylosis (Table 4) have a higher incidence of extremity anomalies (88%), microglossia (63%), and micrognathia (50%). The incidences of CP (38%) and TMJ ankylosis (13%) were approximately the same for each group. Of note is that 63% of patients with glossopalatal ankylosis had facial nerve paralysis; this finding supports our hypothesis that lack of orofacial movement favors the formation of adhesions.

Seven cases of syngnathism appear to have a genetic basis and can be classified as 1) cleft lip–CP–lower lip fistula syndrome[62] or 2) CP–lateral synechiae syndrome[17] (Table 6). Both are transmitted in an autosomal dominant fashion, and a critical appraisal of these syndromes suggests that their separation may be artificial: the different features may be the result of variable penetrance and expressivity. This group appears to be distinctly different from the nonhereditary cases of syngnathism; no cases were associated with microglossia or extremity anomalies. However, significant incidences of CP (100%), cleft lip (85%), lower lip sinus (57%), ankyloblepharon (29%), and TMJ ankylosis (29%) were found. Similarities with Wildervanck's syndrome exist.

Severe syngnathism and glossopalatal ankylosis may lead secondarily to restricted movement and disuse of the TMJ. Although often reported as congenital TMJ ankylosis, these cases of fibrous TMJ ankylosis (Cosack,[25] Davis,[30] Burket,[31] Snijman and Prinsloo,[37] Sarikayalar et al,[41] Narasimharao et al,[43] Sternberg et al,[50] Traaholt,[51] and our case 2) should be differentiated from the less common, isolated cases of true primary ankylosis that occur during the 12th to 18th weeks.

CONCLUSION

It seems likely that there is a link between the posterior BPM group and cases of syngnathism and glossopalatal ankylosis. In the simplest of terms, cases of syngnathism and glossopalatal ankylosis are just variations of anomalous adhesion of adjacent epithelial surfaces; they are not BPM remnants. Similarly, the more heterogeneous cases reported to be anterior BPMs or SGPMs probably also represent anomalous fusion, and not vestigial BPM. This distinction is supported by other physical findings. Cases of posterior BPM are not associated with extremity deformities. If one excludes hereditary syngnathism, in the remaining groups limb abnormalities are found. Since oral development and limb bud proliferation occur concurrently (6th to 10th weeks), global teratogenic or intrauterine events could affect both.

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VESTIBULAR AND BALANCE REHABILITATION THERAPY

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A prospective observational study of the performance of patients enrolled in an individually customized program of habituation and balance rehabilitation physical therapy was conducted from January 1988 to January 1990. Patients ranged in age from 20 to 89 years, with a wide variety of diagnoses. Two global outcome measures — posttherapy symptom response score and pretherapy and posttherapy disability score — were developed and used to judge overall patient performance. In addition, two specific indicators — one for balance performance (dynamic posturography) and one for sensitivity to rapid head movements (motion sensitivity quotient) — were used to measure performance in these two areas. Results indicate statistically significant changes before versus after therapy for both specific measures, and 80% to 85% of the patients showed a reduction in symptoms and disability score following therapy. Analysis of variance and multiple regression analysis indicate that nature of symptoms, pretherapy disability level, history of head injury, and results on dynamic posturography were the variables most predictive of therapy outcome, while age and duration of symptoms made no difference. Other variables, such as medications and site of lesion, were found to affect the length of therapy but not the outcome.

KEY WORDS — balance, dynamic posturography, motion sensitivity quotient, vestibular rehabilitation.

INTRODUCTION

In previously published reports,[1-4] preliminary results of the effectiveness of habituation and balance retraining physical therapy[5] for a wide variety of balance disorder patients were presented. The results suggested that this form of management for balance disorder patients is relatively safe and effective. The findings also implied that the technique was applicable to patients regardless of diagnosis or involvement of the central vestibular system, in addition to the peripheral. The previous results also indicated that patients with a history of head injury as a precipitating event and poor postural control tended to have a poorer prognosis for therapy outcome than others.[2]

The purpose of this paper is to 1) present results of a vestibular rehabilitation management program in 152 patients, studied in a prospective observational manner over a 2-year interval, 2) present a detailed review of performance for head injury patients, and 3) document the impact of such a program on a clinical neurotologic practice.

METHODS

Patient Population. A prospective observational study using consecutive patients evaluated for balance disorders was conducted from January 1988 through December 1989. All patients completed electronystagmography, advanced oculomotor and visual-vestibular interaction testing, rotation chair testing, dynamic posturography,[6] and a neuro-otologic evaluation by a physician. Referral to the rehabilitation program was recommended if the patient's condition was characterized by one or more of the following features:

1. History of positional or motion-provoked symptoms.
2. Evidence of abnormal postural control as demonstrated by dynamic posturography.
3. Indications of an uncompensated peripheral and/or central vestibular system lesion identified physiologically by electronystagmography and/or rotation chair testing and documented functionally by dynamic posturography.

Preliminary work indicated that vestibular rehabilitation therapy was unsuccessful in patients with only spontaneously occurring events of disequilibrium. If other symptoms related to changes in head position or postural control were present between the spontaneous spells, preliminary findings suggested therapy was unsuccessful if the spontaneous events were more frequent than once per month. Therefore, patients with spontaneously occurring spells of vertigo were included if they also had one or more of the three above features and the frequency of spontaneous events was no greater than once per 4 weeks. No attempt was made to exclude patients on the basis of diagnosis, disease severity, or symptom duration. The decision to participate in the therapy program

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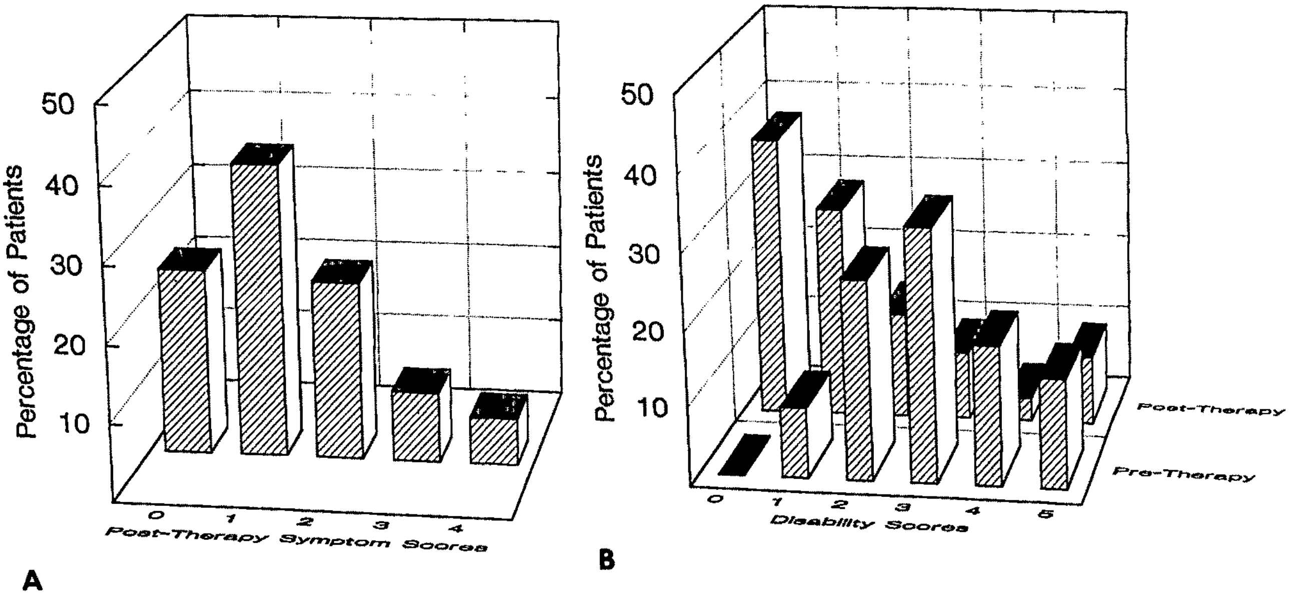

Fig 1. Distribution of scores for 152 patients. A) Posttherapy symptom scores, as defined in text, indicating percentage of patients for each score. B) Percentage of patients for each of disability rating scores, shown for pretherapy and posttherapy ratings.

was left to the discretion of the managing physician and the patient.

Within the 2-year interval of the study, 152 patients (63 men and 89 women) entered and completed active therapy, after which they were enrolled in a posttherapy maintenance program. The patients ranged in age from 20 to 89 years, with a mean age of 52 (SD 16 years). No patient had symptoms for less than 2 months consecutively, with the mean length of time 5 years (SD 5 years). The length of active therapy was never less than 2 weeks, with a mean of 10 weeks (SD 6 weeks). Interpretation of the balance function studies relative to site of lesion and medical diagnosis[2] implying site of lesion indicated that 58% of the patients had unilateral peripheral disease, with only 14 patients (9%) having diagnoses of classic benign paroxysmal positional vertigo. Twenty-three percent had mixed peripheral-central vestibular system lesions, 8% had pure central vestibulo-ocular pathway involvement, 6% undetermined disorders, and 5% bilateral peripheral paresis.

Therapy Program and Therapy Outcome Analysis. All patients underwent a complete physical therapy evaluation. The musculoskeletal and neuromuscular systems were examined. Specific functional deficits related to motion-provoked symptoms or to abnormalities of gait or postural control were evaluated. These measures, together with dynamic posturography, were used to develop individual customized therapy exercise programs that were taught to the patients for home use, with telephone calls and infrequent return visits for follow-up. Exercises were to be done twice a day, once in the morning and once in the evening, unless otherwise instructed. For the small percentage of patients who were felt to be unsafe or otherwise unable to carry out a home program, regular outpatient therapy appointments were initiated. As progress in therapy plateaued, patients were switched from customized exercises to a maintenance program. This program allowed the patient to choose from a wide variety of motion-oriented activities to maintain the benefits derived from customized therapy. The therapy exercises were oriented toward 1) reduction or elimination of motion-provoked and/or positional sensitivity (habituation therapy), 2) correction of functional deficits of balance and gait, and 3) initiation of a general conditioning program. A detailed discussion of the evaluation techniques and the development of the individualized therapy programs used in this study has been published elsewhere and will not be repeated here.[7]

The therapy outcome measures consisted of two global measures (disability rating score and posttherapy symptom score) and two specific measures — one related to postural control (dynamic posturography) and one indicative of changes in sensitivity to rapid positioning (motion sensitivity quotient). Detailed discussions of each of these are found elsewhere,[2,7] and only the definitions for the two global measures will be repeated. A disability rating was given to each patient before and after therapy by the authors. The ratings were defined as follows.

0 No disability; negligible symptoms.
1 No disability; bothersome symptoms.
2 Mild disability; performs usual work duties, but symptoms interfere with outside activities.
3 Moderate disability; symptoms disrupt perfor-

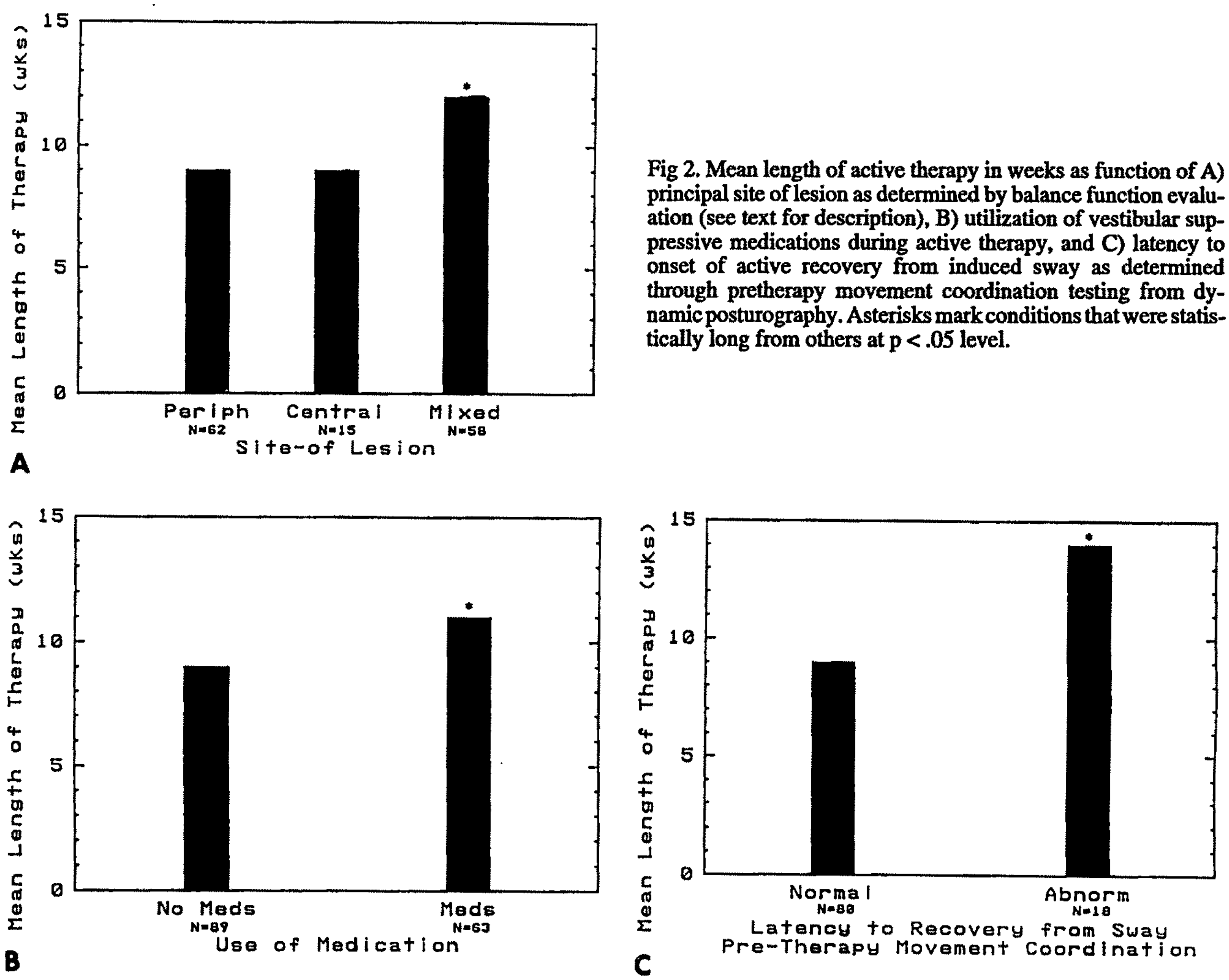

Fig 2. Mean length of active therapy in weeks as function of A) principal site of lesion as determined by balance function evaluation (see text for description), B) utilization of vestibular suppressive medications during active therapy, and C) latency to onset of active recovery from induced sway as determined through pretherapy movement coordination testing from dynamic posturography. Asterisks mark conditions that were statistically long from others at $p < .05$ level.

mance of both usual work duties and outside activities.

4 Recent severe disability; on medical leave or had to change job because of symptoms.

5 Long-term severe disability; unable to work for over 1 year or established permanent disability with compensation payments.

A posttherapy symptom score was assigned to each patient at the time he or she was placed on a maintenance program. The score was determined in response to a series of specific questions that had the patient compare symptoms at the end of active therapy to those prior to therapy. The scores were defined as follows.

0 No symptoms remaining at the end of therapy.

1 Marked improvement in symptoms, mild symptoms remaining.

2 Mild improvement, definite persistent symptoms remaining.

3 No change in symptoms relative to pretherapy period.

4 Symptoms worsened with therapy activities on a persistent basis relative to pretherapy period.

Statistical analysis for comparison of individual variables used a two-tailed standard Student's *t* test. Additionally, multiple analysis of variance and linear regression techniques were used. Statistical significance was defined as $p \leq .05$.

RESULTS

Overall Performance. Figure 1 presents the results from the global indicators of performance: posttherapy symptom scores (Fig 1A) and comparison of pretherapy and posttherapy disability scores (Fig 1B). As shown in Fig 1A, 85% of the patients (addition of patients with scores of 0, 1, and 2) had at least some reduction in symptoms posttherapy compared with what was experienced pretherapy. A small percentage of the patients showed either no change in symptoms or worsening with the therapy exercise program (9% and 6%, respectively). Whenever the symptoms increased on a progressive basis, all had their symptoms return to pretherapy levels once active therapy was stopped. Figure 1B shows the change in distribu-

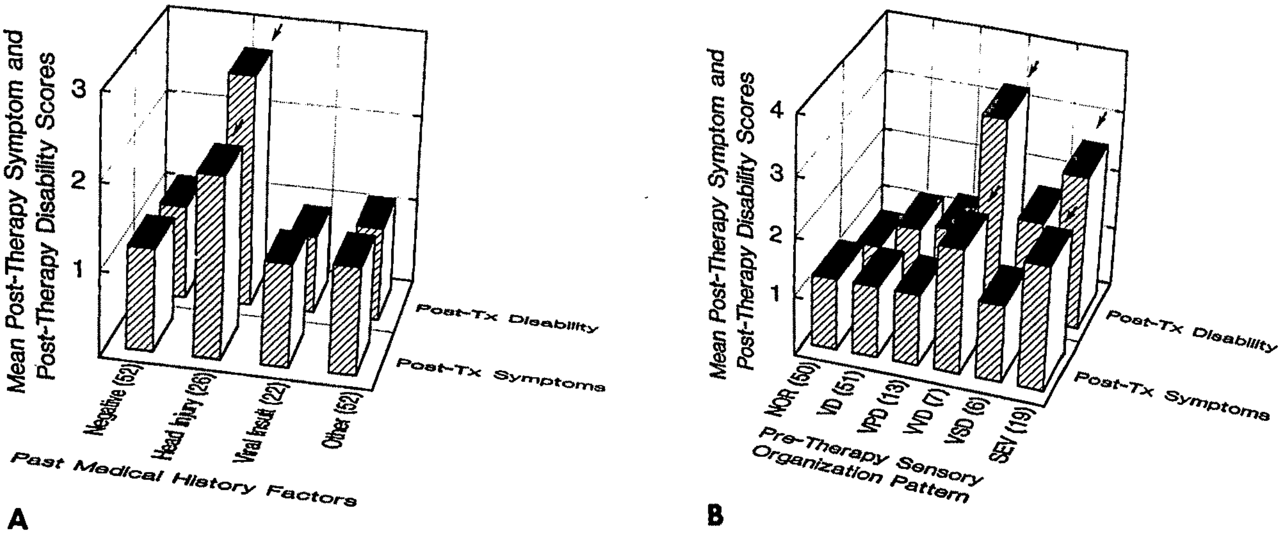

Fig 3. Mean posttherapy symptom scores and posttherapy disability scores. Arrows — significantly different ($p < .05$) from other scores. A) For each of four medical history factors. Numbers in parentheses indicate number of patients reporting each history factor. B) As function of pretherapy sensory organization pattern from posturography. Patterns are defined as follows: NOR — normal, VD — vestibular dysfunction indicating abnormal postural sway when using vestibular system information as only input cue, VPD — visual preference and vestibular dysfunction indicating abnormal postural sway when presented with visually inaccurate information or when relying chiefly on vestibular inputs, VVD — visual and vestibular dysfunction indicating abnormal postural sway when using vestibular system input even when accurate visual cues are present, VSD — somatosensory and vestibular dysfunction indicating abnormal postural sway when using vestibular cues with accurate foot support surface cues, SEV — severe, indicating abnormal sway on four or more of six test conditions that do not meet definition for any of other specified patterns above.

tion of the disability scores from pretherapy to posttherapy. The mean posttherapy disability score (1.4) was significantly lower than the pretherapy score (3.0) at $p < .001$. No patient showed an increase in disability score posttherapy relative to the pretherapy level. While 80% of the patients decreased their disability score by at least 1 point after therapy, the patients that showed the most resistance to change were those with a pretherapy disability score of 5. This was in contrast to those patients with a pretherapy score of 4, in whom a substantial reduction in disability was achieved.

With the two global indicators, the effect of various independent variables on outcome was reviewed for statistical significance. No effect on final outcome was noted for site of lesion (peripheral, central, or mixed peripheral-central, as indicated by balance function testing and final diagnosis), concomitant use of vestibular suppressive medication, or abnormalities in the movement coordination portion of dynamic posturography (measured latency reactions to induced forward or backward sway). However, each of these parameters showed significant impact ($p \leq .05$) on the length of therapy. Therapy was longer for those patients with mixed lesion sites, those taking vestibular suppressive medications, and those with indications of prolonged latencies for recovery from induced forward or backward sway (Fig 2).

Figure 3 demonstrates that medical history and pretherapy posturography had statistically significant effects on overall therapy outcome. The category of "other" includes 52 patients having a variety of other factors as their only predisposing history findings, with too few persons in any one category for analysis. The patients with a history of head injury (with or without loss of consciousness) had significantly poorer outcomes by both measures at the $p < .004$ level. In Fig 3B, the effect of pretherapy dynamic posturography sensory organization patterns on therapy outcome shows significantly ($p < .05$) poorer results for those patients having visual-vestibular dysfunction or severe dysfunction patterns.

The effects of age and gender are shown in Fig 4. While the 40- to 59-year-old group appears to show poorer performance, the only statistically significant age difference was between this age group and that over 70 years old (Fig 4A). It should be noted that the number of head injury patients was 13 (21%) in the 40- to 59-year-old category and only 1 (4%) in the over-70 group. The posttherapy disability score for men was significantly poorer than for women ($p = .002$); however, there was no significant difference in pretherapy disability scores for men versus women. The posttherapy symptom scores were not found to be significantly different (Fig 4B).

Overall performance for those patients with head injury as an antecedent event (N = 26) is shown in Fig 5. The percentage of patients with some reduction in

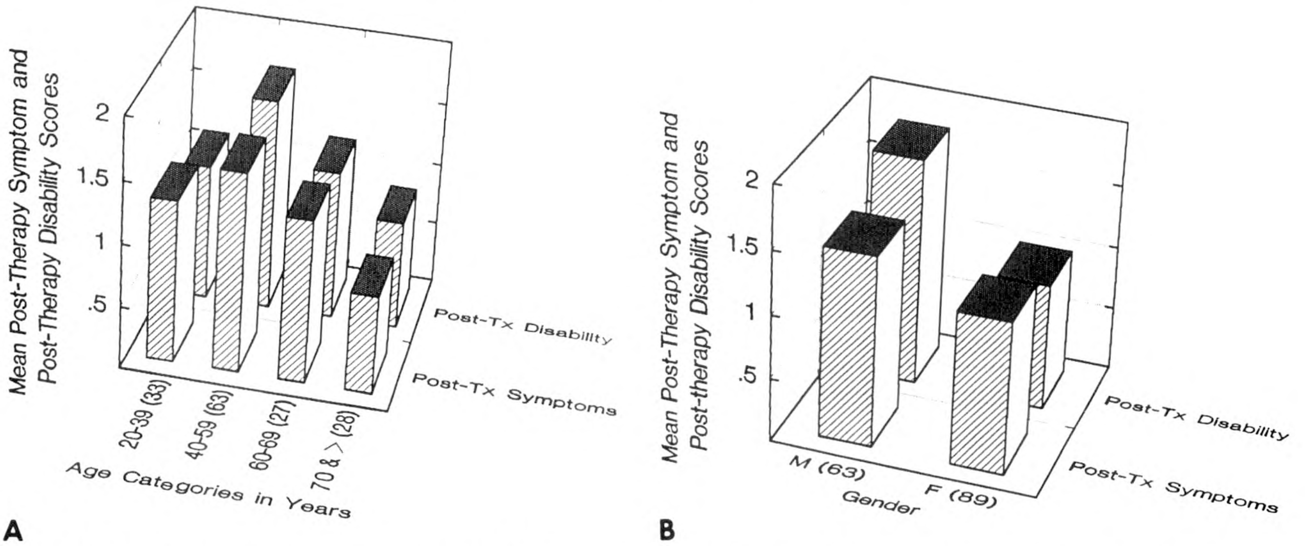

Fig 4. Mean posttherapy symptom scores and disability scores, shown as function of A) age groupings and B) gender of patients. Number of patients in each age or gender category is given in parentheses.

symptoms was substantially lower (62%), and the percentage of patients showing no change or becoming worse was significantly higher than the total population of 152 patients (compare to Fig 1A). The pretherapy and posttherapy disability score profiles (Fig 5B) present an altered pattern compared with the full group (Fig 1B). The major difference was the increase in posttherapy disability score 3 and the increase in both pretherapy and posttherapy disability score 5 for the head injury group. Only 53% of the head injury patients reduced their disability score by at least one point, compared to 80% of the total group.

Prior to the use of rehabilitation therapy, 27 patients with uncompensated vestibular neuritis (1983 to 1986) were treated in our department with retrolabyrinthine vestibular nerve sections. A reduction in symptoms was achieved for 74% of these patients, with 22% showing no change and 1 patient (4%) becoming worse.[8] For this same diagnostic category, 22 patients were seen over the period of this study, with 86% showing reduction in symptoms, 14% showing no change, and no patient becoming worse.

Specific Performance. Pretherapy and posttherapy comparison of the mean equilibrium composite score (N = 82) for the sensory organization portion of dynamic posturography showed a significant improvement ($p = .003$). This difference may not represent a functionally significant change, since both pretherapy and posttherapy mean composite scores were very near the normal range. In fact, a small portion of the change may be related to a minor learning effect that has been noted in randomly sampled balance-disordered patients when testing is repeated on the same day.[9] The posttherapy mean composite score for those patients not using suppressive medication was statistically greater (indicating better performance) than the posttherapy mean composite score for posturography for those using suppressive medication during therapy. This difference related to medication usage represents a functional difference, since the group taking medication showed a posttherapy mean composite posturography score less than normal, while those without medication showed a mean composite score above normal. A statistically significant ($p < .001$) improvement was seen in the mean motion sensitivity quotient[2,7] for the posttherapy (75 patients) compared to the pretherapy evaluation.

Predicting Therapy Outcome. The independent variables showing consistent, statistically significant prediction abilities (by multiple regression techniques) for both posttherapy symptom scores and posttherapy disability scores were used to develop profiles of patients according to their overall performance in the therapy program. Overall performance was classified as follows.

1. Excellent: posttherapy disability score of 0 or 1, and posttherapy symptom score of 0 or 1 (89 patients).
2. Fair: posttherapy disability score of 2 or 3, and posttherapy symptom score of 2 (23 patients).
3. Poor: posttherapy disability score of 4 or 5, and posttherapy symptom score of 3 or 4 (13 patients).

Figure 6 shows the percentage of patients displaying each of the independent variable factors for each classification of overall therapy outcome.

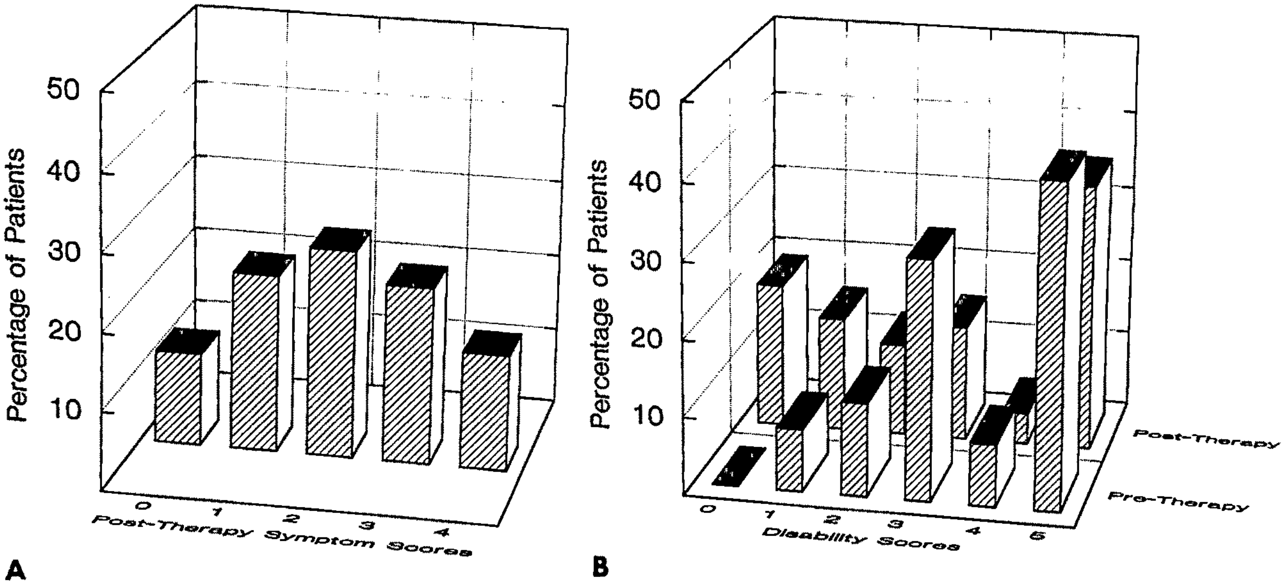

Fig 5. Histograms for patients with history of head injury prior to symptom onset. Compare with Fig 1. A) Posttherapy symptom scores. B) Disability scores.

DISCUSSION

As suggested by our previously published preliminary results, the present data continue to indicate that vestibular rehabilitation therapy is an important and effective management strategy for patients with chronic balance disorders. The program is applicable to a wide variety of diagnoses, lesion site combinations, and ages, yet there are certain characteristics that suggest an increased likelihood for good overall therapy outcome (Fig 6). While certain patient profiles may predict the relative effectiveness of this management technique, it is not appropriate at this time to exclude from therapy a patient who might benefit. More important, the profiles associated with poorer outcomes imply the need for improvements in our therapy program to better manage these cases. This is especially true for head injury patients. With some of these patients, concomitant cognitive, psychologic, and physical sequelae of closed head injury may restrict compliance with a home-based therapy program.

In addition to head injury history, the nature of patient symptoms, posturography findings, and pretherapy disability score can be used as prognostic indicators. This seems especially true if a pretherapy disability score of 5 is noted. The percentage of patients in the overall group (N = 152) and in the head injury group (N = 26) who were able to go from a disability score of 5 to one of 3 or less was small (5% in the total group and 7% in the head injury group; Figs 1B and 5B, respectively). The only difference between scores of 4 and 5 was the length of time the patient was disabled, yet the ability to change from a pretherapy score of 4 to a posttherapy score of 3 or less was substantially greater than for patients scoring a 5. There may be a critical time period within which some individuals may achieve significant functional improvement and return to the work force. This time period may be influenced by negative psychosocial forces that develop when an individual is placed on permanent disability with financial compensation. The lack of an adverse effect on therapy outcome by length of symptoms in the total group, together with the fact that those with pretherapy disability scores less than 4 with continuous symptoms for years were able to achieve significant improvement, argues against a critical period for therapy responsiveness in mild cases.

Posturography was used globally as one indicator of patient candidacy for therapy. The detailed pattern of the pretherapy sensory organization test was found by regression analysis to be a prognostic indicator of overall therapy performance. While these are not independent, the posturography criterion for inclusion does not appear to influence the prognostic indications from posturography evaluation. This is demonstrated by the pretherapy pattern's continuing to be a prognostic indicator (shown via regression analysis) when patients for whom posturography was not a primary criterion for inclusion were reviewed.

The relative safety and significant success rate of the therapy program for treatment of uncompensated vestibular neuritis patients (at only a fraction of the cost of retrolabyrinthine vestibular nerve section) has altered our management of these patients. During the 2 years of this retrospective review, nerve sections were performed primarily for Meniere's disease, and the patients with uncompensated vestibular neuritis

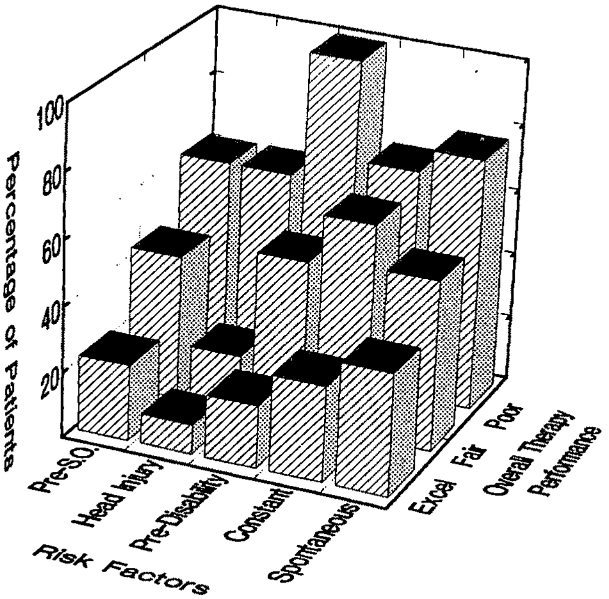

Fig 6. Percentage of patients within each of overall therapy performance categories (excellent, fair, or poor as defined in text) displaying each of following pretherapy characteristics. Spontaneous — spontaneous symptoms of vertigo or lightheadedness/unsteadiness, Constant — continuous symptoms of vertigo or lightheadedness/unsteadiness, Pre-Disability — pretherapy disability rating score of 4 or 5, Head Injury — history of head injury as precipitating event to symptoms, Pre-S.O. — pretherapy sensory organization patterns from dynamic posturography *other than* normal or vestibular dysfunction (see legend of Fig 3).

were managed by therapy instead. Our management of patients suspected of perilymphatic fistula has also changed. When no hearing complications are evident, therapy is instituted prior to an exploratory procedure to assist in selecting those with truly unstable peripheral labyrinthine function. The patients who become worse during therapy are most likely to benefit from surgery.[10]

An observational study of the type reported here, while strongly encouraging, leaves many important questions unanswered. Since no control group was used, the benefit of individually customized therapy programs over generic vestibular exercises has yet to be proven. We hypothesize that there may be subgroups of individuals who would respond equally well to either a generic or a customized program. This issue is currently being addressed with a 3-year prospectively randomized trial. Without an untreated control group it is also difficult to evaluate whether spontaneous resolution of symptoms would have occurred in some of our patients. However, a large spontaneous remission rate is not likely, given the presence of continuous symptoms for several months, and in most cases, years.

A recent randomly sampled review of the long-term effects of the vestibular rehabilitation program indicates that the immediate therapy outcome is maintained in 86% of the patients sampled.[11] This argues against any significant placebo effect to the therapy.

CONCLUSIONS

1. The 2-year review strongly indicates that the physical therapy rehabilitation program is an efficient, relatively safe, and effective management strategy for a wide variety of balance disorder patients.

2. The program appears to be applicable for patients of all ages with minimal gender differences.

3. Prognostic indicators for therapy outcome may be useful in counseling and in developing realistic goals and referral priorities, but are not used for excluding patients from therapy at this time.

4. Patients with spontaneous and/or continuous symptoms of disequilibrium, a head injury history, permanent disability, and/or severe postural control abnormalities perform the poorest in the current therapy program. More intensive interventions may be required to optimize the effectiveness of rehabilitation for these individuals.

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Ann Otol Rhinol Laryngol 102:1993

CUTANEOUS SQUAMOUS CELL CARCINOMA OCCURRING IN A PECTORALIS MAJOR MYOCUTANEOUS FLAP

CASE REPORT AND LITERATURE REVIEW

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The pectoralis major myocutaneous flap is a reliable regional flap commonly used for the reconstruction of head and neck defects. We report a case of a squamous cell carcinoma that occurred on the cutaneous paddle of a pectoralis flap in a patient with previous chest wall lesions. The need for a careful history of previous chest wall cutaneous lesions is stressed in patients who are to undergo this type of reconstruction.

KEY WORDS — mouth neoplasms, squamous cell carcinoma, surgical flaps.

INTRODUCTION

The pectoralis major myocutaneous flap is a reliable regional flap that is commonly used for reconstruction of head and neck defects. It allows the transfer of large amounts of chest wall skin, subcutaneous tissue, and muscle in a single stage reconstruction. Its popularity among head and neck reconstructive surgeons is primarily due to its ease of elevation, high survival rate, and low complication rate.

This paper presents a case of the development of a cutaneous squamous cell carcinoma on the skin paddle of a pectoralis major myocutaneous flap in a patient with a history of previous chest wall lesions (squamous cell carcinoma). To our knowledge, this is a previously unreported complication.

CASE REPORT

This patient presented in 1982 at the age of 52 years with a lesion of the left anterior floor of the mouth. He had a significant history for tobacco and ethanol use. His surgical history was remarkable for previous excisions of right temporal and left chest "skin cancers." He had significant sun exposure during his youth.

Evaluation revealed him to have a T3N0M0 squamous cell carcinoma of the left anterior floor of the mouth. This was treated with 65 Gy to the primary and regional draining nodes. He stopped his use of tobacco at this time, but continued to have two alcoholic drinks per night.

In December 1982, this lesion recurred and he was referred to us for further treatment. He subsequently underwent a resection of the left floor of the mouth and the oral tongue, a marginal mandibulectomy, bilateral suprahyoid neck dissections, and reconstruction with a left pectoralis major myocutaneous flap. Included in the flap reconstruction was a 5 x 6-cm cutaneous paddle. Pathology was remarkable for an ulcerated squamous cell carcinoma, keratinizing and invasive, arising at the junction of the tongue and the floor of the mouth. The margins were free of tumor, and the patient recovered well.

In April 1985 he developed a lesion on his right anterior chest wall. This was excised and diagnosed as well-differentiated squamous cell carcinoma.

In December 1990 he presented again, this time with multiple lesions involving the left auricle, the left upper lip, and the left floor of mouth flap reconstruction. Intraoral examination revealed a 1-cm lesion on the cutaneous portion of the pectoralis flap (Fig 1). This was excised and pathologic examination showed that it was a squamous cell carcinoma, well to moderately differentiated, keratinizing and invasive. The epidermis adjacent to the lesion was noted to have actinic changes, considered a precursor to cutaneous squamous cell carcinoma (Fig 2). The auricular lesion was also remarkable for squamous cell carcinoma, well differentiated, for which he underwent further excision followed by radiotherapy. The lip lesion was excised and pathologic examination revealed it to be solar keratosis. At last follow-

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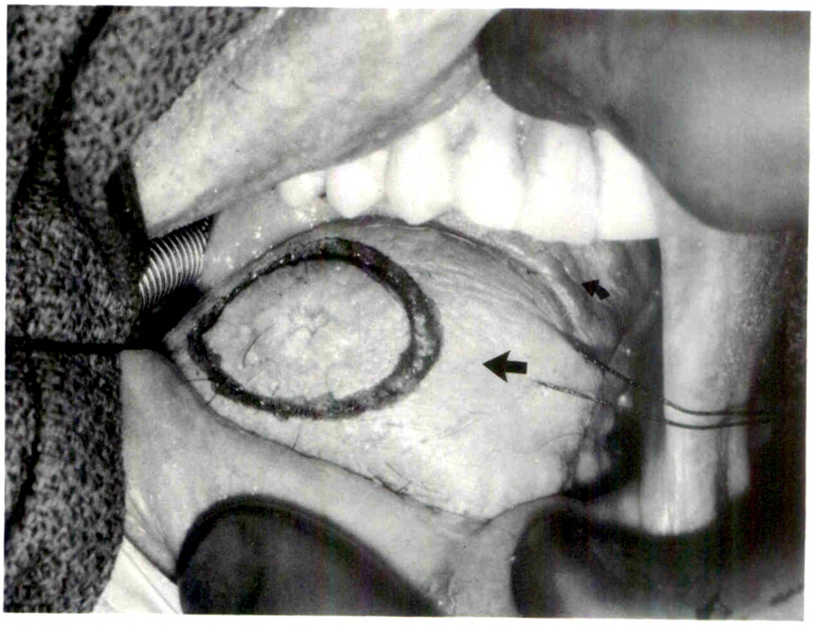

Fig 1. Ulcerated squamous cell carcinoma on pectoralis flap used for intraoral reconstruction. Lesion has been outlined circumferentially with carbon dioxide laser. Large arrow — pectoralis flap, small arrow — tongue.

up, he had no evidence of recurrent squamous cell carcinoma in the oral cavity, auricle, or chest wall. The floor of the mouth reconstruction has healed uneventfully and he is able to wear a lower denture.

DISCUSSION

The pectoralis major myocutaneous flap is a reliable means of regional transfer of skin, muscle, and subcutaneous tissue for reconstruction of head and neck defects. It has low failure and complication rates.[1,2] The complication presented in this case report has not been previously reported.

Maas et al[3] presented a case report of a woman who underwent pectoralis flap reconstruction of an oropharyngeal defect following composite resection. Intraoperatively it was noted that there were cystic masses within the transferred breast tissue. Frozen section analysis revealed only fibrocystic disease; however, the permanent sections showed there to be lobular carcinoma in situ. The patient received postoperative chemotherapy and radiotherapy. At 2 years postoperatively there was no evidence of recurrent disease, and mammograms were negative. Maas et al recommended that women who are to undergo pectoralis major flap reconstruction have careful breast examinations including mammography. There should be a liberal use of intraoperative frozen section analysis of suspicious breast lesions, and an alternate plan of reconstruction should be available if breast carcinoma is found.

Robbins and Woodson[4] reported two cases of chest wall metastasis as a complication of pectoralis flap reconstruction. Both patients had undergone resection of recurrent oropharyngeal carcinoma and reconstruction with a pectoralis major myocutaneous flap. Both patients subsequently developed metastatic squamous cell carcinoma at the flap donor site. The authors hypothesized that this occurred because of tumor seeding at the time of surgery. To prevent this complication it was recommended that the wound

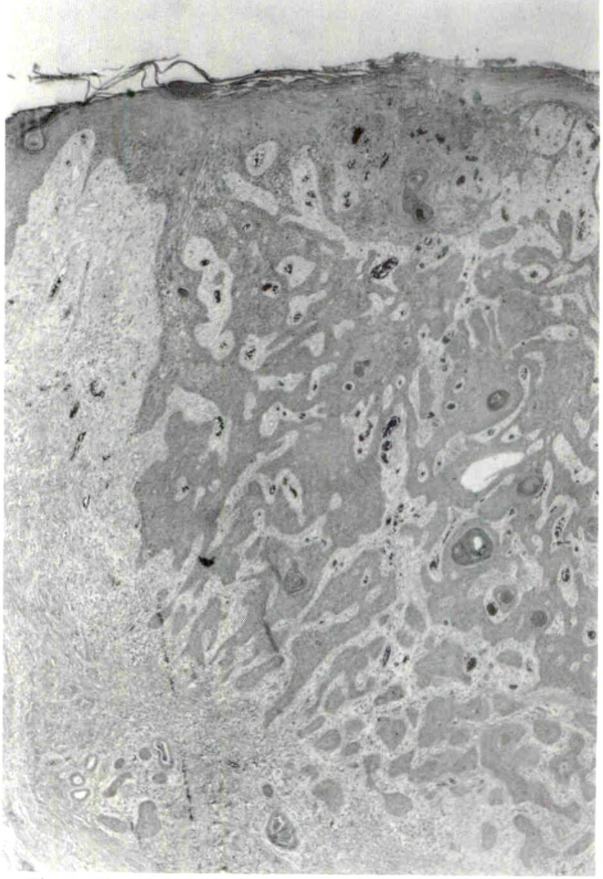

Fig 2. Photomicrograph of lesion demonstrating keratinizing invasive squamous cell carcinoma. Dermal elements are clearly seen. Epidermis adjacent to lesion is remarkable for actinic changes.

be thoroughly irrigated and that instruments, gowns, and gloves be changed after ablation and prior to starting the reconstruction.

Ellison et al[5] presented three cases of tumor recurrence within myocutaneous flaps. These patients underwent resection of primary lesions of the hypopharynx (one) and oropharynx (two). They were reconstructed with a pectoralis major myocutaneous flap (two, with one failure), a trapezius myocutaneous flap (one), and a sternocleidomastoid myocutaneous flap (one). All three patients developed recurrent squamous cell carcinoma at the base of the flap pedicle. The authors suggested that these lesions were from lymphatic dissemination into the flap pedicle or from tumor implantation at the time of surgery.

Badellino et al[6] presented a case of chest wall metastasis secondary to pectoralis flap transfer. Their patient had undergone composite resection for recurrent squamous cell carcinoma following irradiation, chemotherapy, and surgery for an oral cavity carcinoma. Soft tissue reconstruction was performed with a pectoralis major myocutaneous flap. Eight months after surgery, the patient developed recurrent disease adjacent to the intraoral portion of the flap. He was begun on chemotherapy, with a partial response. However, he subsequently developed a nodule of tumor along the transposed flap, with no contiguity with the intraoral recurrence. This was situated at the base of the flap pedicle at the chest wall. He subsequently died of his disease. The authors felt that this represented lymphatic spread along the pectoralis neurovascular pedicle. They suggested careful follow-up with monitoring for distant spread along the vascular pedicle of a flap reconstruction.

The case that we present is clearly different from those discussed above. This patient had a previous history of multiple cutaneous lesions of squamous cell carcinoma that involved his temporal area and chest wall. This patient probably had occult microscopic epithelial changes in his chest wall skin at the time of pectoralis flap reconstruction. He then developed an obvious chest wall lesion that required excision 2 years later and a lesion on the transposed chest wall skin that required excision 8 years following his intraoral reconstruction.

We conclude that this is an extremely rare complication of pectoralis flap reconstruction. However, in patients with a strong history of cutaneous squamous cell carcinoma, particularly of the chest wall, careful examination of the chest skin is essential. Other flap donor sites or the use of the pectoralis muscle as a myofascial flap may be considered, with or without a split thickness skin graft.

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Chung MH, Griffith SR, Park KH, Lim DJ, DeMaria TF. (Columbus, USA) Cytological and histological changes in the middle ear after inoculation of influenza A virus.

Crespo PV, Lopez-Escamez JA, Canizares FJ, Campos A. (Granada, Spain) X-ray microanalytical determination of P, S and K concentrations in the gelatinous membrane of the utricle.

Ikeda K, Sunose H, Takasaka T. (Sendai, Japan) Effects of free radicals on the intracellular calcium concentration in the isolated outer hair cell of the guinea pig cochlea.

A Selection of Recently Published and Forthcoming Supplements

499: *Iurato I, ed.* Multicentre evaluation of the temporal bones obtained from a patient with suspected Meniere's disease.

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INDICATIONS AND USAGE VANCENASE AQ Nasal Spray is indicated for the relief of the symptoms of seasonal or perennial allergic and non-allergic (vasomotor) rhinitis. Results from two clinical trials have shown that significant symptomatic relief was obtained within three days. However, symptomatic relief may not occur in some patients for as long as two weeks. VANCENASE AQ Nasal Spray should not be continued beyond three weeks in the absence of significant symptomatic improvement. VANCENASE AQ Nasal Spray should not be used in the presence of untreated localized infection involving the nasal mucosa.

VANCENASE AQ Nasal Spray is also indicated for the prevention of recurrence of nasal polyps following surgical removal.

Clinical studies have shown that treatment of the symptoms associated with nasal polyps may have to be continued for several weeks or more before a therapeutic result can be fully assessed. Recurrence of symptoms due to polyps can occur after stopping treatment, depending on the severity of the disease.

CONTRAINDICATIONS Hypersensitivity to any of the ingredients of this preparation contraindicates its use.

WARNINGS The replacement of a systemic corticosteroid with VANCENASE AQ Nasal Spray can be accompanied by signs of adrenal insufficiency.

When transferred to VANCENASE AQ Nasal Spray, careful attention must be given to patients previously treated for prolonged periods with systemic corticosteroids. This is particularly important in those patients who have associated asthma or other clinical conditions, where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate day prednisone systemic treatment and orally inhaled beclomethasone increased the likelihood of HPA suppression compared to a therapeutic dose of either one alone. Therefore, VANCENASE AQ Nasal Spray treatment should be used with caution in patients already on alternate day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, VANCENASE AQ Nasal Spray should be discontinued slowly, consistent with accepted procedures for discontinuing oral steroid therapy.

Children who are on immunosuppressant drugs are more susceptible to infections than healthy children. Chickenpox and measles, for example, can have a more serious or even fatal course in children on immunosuppressant corticosteroids. In such children, or in adults who have not had these diseases, particular care should be taken to avoid exposure. If exposed, therapy with varicella zoster immune globulin (VZIG) or pooled intravenous immunoglobulin (IVIG), as appropriate, may be indicated. If chickenpox develops, treatment with antiviral agents may be considered.

PRECAUTIONS **General:** During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, eg, joint and/or muscular pain, lassitude and depression. Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone.

Extremely rare instances of wheezing, nasal septum perforation and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids. Although these have not been observed in clinical trials with VANCENASE AQ Nasal Spray, vigilance should be maintained.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuance of treatment with VANCENASE AQ Nasal Spray.

If persistent nasopharyngeal irritation occurs, it may be an indication for stopping VANCENASE AQ Nasal Spray.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of VANCENASE AQ Nasal Spray may suppress HPA function.

VANCENASE AQ Nasal Spray should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract, or in untreated fungal, bacterial, systemic viral infections, or ocular herpes simplex.

For VANCENASE AQ Nasal Spray to be effective in the treatment of nasal polyps, the spray must be able to enter the nose. Therefore, treatment of nasal polyps with VANCENASE AQ Nasal Spray should be considered adjunctive therapy to surgical removal and/or the use of other medications which will permit effective penetration of VANCENASE AQ Nasal Spray into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using VANCENASE AQ Nasal Spray over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septal ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses should be avoided.

Information for Patients: Patients being treated with VANCENASE AQ Nasal Spray should receive the following information and instructions. This information is intended to aid in the safe and effective use of medication. It is not a disclosure of all possible adverse or intended effects. Patients should use VANCENASE AQ Nasal Spray at regular intervals since its effectiveness depends on its regular use. The patient should take the medication as directed. It is not acutely effective and the prescribed dosage should not be increased. Instead, nasal vasoconstrictors or oral antihistamines may be needed until the effects of VANCENASE AQ Nasal Spray are fully manifested. One to two weeks may pass before full relief is obtained. The patient should contact the physician if symptoms do not improve, or if the condition worsens, or if sneezing or nasal irritation occurs. For the proper use of this unit and to attain maximum improvement, the patient should read and follow the accompanying Patient's Instructions carefully.

Patients who are on immunosuppressant doses of corticosteroids should be warned to avoid exposure to chickenpox or measles and, if exposed, to obtain medical advice.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrous cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrous cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy Category C: Like other corticosteroids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately ten times the human dose. In these studies beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at ten times the human dose or orally at 1000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when VANCENASE AQ Nasal Spray is administered to nursing women.

Pediatric Use: Safety and effectiveness in children below the age of 6 years have not been established.

ADVERSE REACTIONS In general, side effects in clinical studies have been primarily associated with irritation of the nasal mucous membranes. Rarely, immediate hypersensitivity reactions may occur after the intranasal administration of beclomethasone dipropionate.

Adverse reactions reported in controlled clinical trials and open studies in patients treated with VANCENASE AQ Nasal Spray are described below.

Mild, transient nasopharyngeal irritation following the use of beclomethasone aqueous nasal spray has been reported in up to 24% of patients treated, including occasional sneezing attacks (about 4%) occurring immediately following use of the inhaler. In patients experiencing these symptoms, none had to discontinue treatment. The incidence of irritation and sneezing was approximately the same in the group of patients who received placebo in these studies, implying that these complaints may be related to vehicle components of the formulation.

Fewer than 5 per 100 patients reported headache, nausea or lightheadedness following the use of VANCENASE AQ (beclomethasone dipropionate, monohydrate) Nasal Spray. Fewer than 3 per 100 patients reported nasal stuffiness, nosebleeds, rhinorrhea, and tearing eyes.

Extremely rare instances of wheezing, nasal septum perforation and increased intraocular pressure have been reported following the intranasal administration of aerosolized corticosteroids (see **PRECAUTIONS**).

OVERDOSAGE When used at excessive doses, systemic corticosteroid effects such as hypercorticism and adrenal suppression may appear. If such changes occur, VANCENASE AQ Nasal Spray should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy. The oral LD_{50} of beclomethasone dipropionate is greater than 1 g/kg in rodents. One bottle of VANCENASE AQ Nasal Spray contains beclomethasone dipropionate, monohydrate equivalent to 10.5 mg of beclomethasone dipropionate; therefore, acute overdosage is unlikely.

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POCKETHALER® Nasal Inhaler

For Nasal Inhalation Only

BRIEF SUMMARY

(For full Prescribing Information see package insert)

INDICATIONS AND USAGE VANCENASE POCKETHALER Nasal Inhaler is indicated for the relief of the symptoms of seasonal or perennial rhinitis in those cases poorly responsive to conventional treatment.

VANCENASE POCKETHALER Nasal Inhaler is also indicated for the prevention of recurrence of nasal polyps following surgical removal.

Clinical studies in seasonal and perennial rhinitis have shown that improvement is usually apparent within a few days. However, symptomatic relief may not occur in some patients for as long as 2 weeks. Although systemic effects are minimal at recommended doses, VANCENASE treatment should not be continued beyond 3 weeks in the absence of significant symptomatic improvement. VANCENASE treatment should not be used in the presence of untreated, localized infection involving the nasal mucosa.

Clinical studies have shown that treatment of the symptoms associated with nasal polyps may have to be continued for several weeks or more before a therapeutic result can be fully assessed. Recurrence of symptoms due to polyps can occur after stopping treatment, depending on the severity of the disease.

CONTRAINDICATIONS Hypersensitivity to any of the ingredients of this preparation contraindicates its use.

WARNINGS The replacement of a systemic corticosteroid with VANCENASE POCKETHALER Nasal Inhaler can be accompanied by signs of adrenal insufficiency.

Careful attention must be given when patients, previously treated for prolonged periods with systemic corticosteroids, are transferred to VANCENASE POCKETHALER Nasal Inhaler. This is particularly important in those patients who have associated asthma or other clinical conditions, where too rapid a decrease in systemic corticosteroids may cause a severe exacerbation of their symptoms.

Studies have shown that the combined administration of alternate day prednisone systemic treatment and orally inhaled beclomethasone increased the likelihood of HPA suppression compared to a therapeutic dose of either one alone. Therefore, VANCENASE treatment should be used with caution in patients already on alternate day prednisone regimens for any disease.

If recommended doses of intranasal beclomethasone are exceeded or if individuals are particularly sensitive or predisposed by virtue of recent systemic steroid therapy, symptoms of hypercorticism may occur, including very rare cases of menstrual irregularities, acneiform lesions, and cushingoid features. If such changes occur, VANCENASE POCKETHALER Nasal Inhaler should be discontinued slowly, consistent with accepted procedures for discontinuing oral steroid therapy.

Children who are on immunosuppressant drugs are more susceptible to infections than healthy children. Chickenpox and measles, for example, can have a more serious or even fatal course in children on immunosuppressant corticosteroids. In such children, or in adults who have not had these diseases, particular care should be taken to avoid exposure. If exposed, therapy with varicella zoster immune globulin (VZIG) or pooled intravenous immunoglobulin (IVIG), as appropriate, may be indicated. If chickenpox develops, treatment with antiviral agents may be considered.

PRECAUTIONS General: During withdrawal from oral steroids, some patients may experience symptoms of withdrawal, eg, joint and/or muscular pain, lassitude, and depression.

Extremely rare instances of nasal septum perforation and increased intraocular pressure have been reported following the intranasal application of aerosolized corticosteroids.

In clinical studies with beclomethasone dipropionate administered intranasally, the development of localized infections of the nose and pharynx with *Candida albicans* has occurred only rarely. When such an infection develops, it may require treatment with appropriate local therapy or discontinuance of treatment with VANCENASE POCKETHALER Nasal Inhaler.

Beclomethasone dipropionate is absorbed into the circulation. Use of excessive doses of VANCENASE POCKETHALER Nasal Inhaler may suppress HPA function.

VANCENASE treatment should be used with caution, if at all, in patients with active or quiescent tuberculous infections of the respiratory tract, or in untreated fungal, bacterial, systemic viral infections, or ocular herpes simplex.

For VANCENASE POCKETHALER Nasal Inhaler to be effective in the treatment of nasal polyps, the aerosol must be able to enter the nose. Therefore, treatment of nasal polyps with VANCENASE Nasal Inhaler should be considered adjunctive therapy to surgical removal and/or the use of other medications which will permit effective penetration of the VANCENASE product into the nose. Nasal polyps may recur after any form of treatment.

As with any long-term treatment, patients using VANCENASE treatment over several months or longer should be examined periodically for possible changes in the nasal mucosa.

Because of the inhibitory effect of corticosteroids on wound healing, patients who have experienced recent nasal septum ulcers, nasal surgery, or trauma should not use a nasal corticosteroid until healing has occurred.

Although systemic effects have been minimal with recommended doses, this potential increases with excessive doses. Therefore, larger than recommended doses should be avoided.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of rats for a total of 95 weeks, 13 weeks by inhalation and 82 weeks by the oral route, resulted in no evidence of carcinogenic activity. Mutagenic studies have not been performed.

Impairment of fertility, as evidenced by inhibition of the estrus cycle in dogs, was observed following treatment by the oral route. No inhibition of the estrus cycle in dogs was seen following treatment with beclomethasone dipropionate by the inhalation route.

Pregnancy Category C: Like other corticoids, parenteral (subcutaneous) beclomethasone dipropionate has been shown to be teratogenic and embryocidal in the mouse and rabbit when given in doses approximately ten times the human dose. In these studies, beclomethasone was found to produce fetal resorption, cleft palate, agnathia, microstomia, absence of tongue, delayed ossification, and agenesis of the thymus. No teratogenic or embryocidal effects have been seen in the rat when beclomethasone dipropionate was administered by inhalation at ten times the human dose or orally at 1000 times the human dose. There are no adequate and well-controlled studies in pregnant women. Beclomethasone dipropionate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects: Hypoadrenalism may occur in infants born of mothers receiving corticosteroids during pregnancy. Such infants should be carefully observed.

Nursing Mothers: It is not known whether beclomethasone dipropionate is excreted in human milk. Because other corticosteroids are excreted in human milk, caution should be exercised when VANCENASE POCKETHALER Nasal Inhaler is administered to nursing women.

Pediatric Use: Safety and effectiveness in children below the age of 6 years have not been established.

ADVERSE REACTIONS In general, side effects in clinical studies have been primarily associated with the nasal mucous membranes. Adverse reactions reported in controlled clinical trials and in long-term open studies in patients treated with VANCENASE Nasal Inhaler are described below.

Sensations of irritation and burning in the nose (11 per 100 patients) following the use of VANCENASE Nasal Inhaler have been reported. Also, occasional sneezing attacks (10 per 100 patients) have occurred immediately following the use of the intranasal inhaler. This symptom may be more common in children.

Rhinorrhea may occur occasionally (1 per 100 patients).

Localized infections of the nose and pharynx with *Candida albicans* have occurred rarely. (See **PRECAUTIONS.**)

Transient episodes of epistaxis or bloody discharge from the nose have been reported in 2 per 100 patients.

Ulceration of the nasal mucosa has been reported rarely.

Extremely rare instances of nasal septum perforation have been reported following the intranasal application of aerosolized corticosteroids. Rare cases of immediate and delayed hypersensitivity reactions, including urticaria, angioedema, rash, and bronchospasm have been reported following the oral and intranasal inhalation of beclomethasone.

Increased intraocular pressure has been reported rarely. (See **PRECAUTIONS.**)

Systemic corticosteroid side effects were not reported during controlled clinical trials. If recommended doses are exceeded, however, or if individuals are particularly sensitive, symptoms of hypercorticism, ie, Cushing's syndrome could occur.

OVERDOSAGE When used at excessive doses, systemic corticosteroid effects such as hypercorticism and adrenal suppression may appear. If such changes occur, VANCENASE POCKETHALER Nasal Inhaler should be discontinued slowly consistent with accepted procedures for discontinuing oral steroid therapy.

The oral LD_{50} of beclomethasone dipropionate is greater than 1 g/kg in rodents. One canister of VANCENASE POCKETHALER Nasal Inhaler contains 8.4 mg of beclomethasone dipropionate; therefore acute overdosage is unlikely.

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ERYTHROMYCIN IN ACUTE LARYNGITIS IN ADULTS

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Moraxella catarrhalis and *Hemophilus influenzae* are isolated from the nasopharynx in 50% to 55% and 8% to 15%, respectively, of cases of acute laryngitis in adults. This finding indicates that these organisms, *M catarrhalis* in particular, are in some way involved in the pathogenesis of the disorder. In the present double-blind, placebo-controlled trial, the effect of erythromycin ethylsuccinate (0.5 g twice a day for 5 days) on the elimination of nasopharyngeal pathogens and reduction of clinical signs of upper respiratory tract infection, as well as on subjective complaints, was evaluated in 106 adults with acute laryngitis. The bacterial isolation rates at presentation were *M catarrhalis* 50%, *H influenzae* 18%, and *Streptococcus pneumoniae* 4%. In the 99 patients who completed the study, the elimination of *M catarrhalis* after 1 week was better in the erythromycin group (25 of 30 cases) than in the placebo group (6 of 19 cases; $p \leq .00038$). The elimination of *H influenzae* was unaffected by erythromycin. Otolaryngologic examination did not reveal any significant group differences regarding laryngitis, pharyngitis, or rhinitis. Voice quality was improved after 1 week, irrespective of treatment. However, as compared to the placebo group, the erythromycin group reported fewer voice complaints after 1 week and fewer coughing complaints after 2 weeks. As acute laryngitis in adults is self-limiting, and subjective symptoms are spontaneously reduced after 1 week in most cases, antibiotic treatment does not seem warranted as a general policy. However, erythromycin may be justified in patients who are professionally dependent on voice function.

KEY WORDS — acute laryngitis, erythromycin, *Moraxella catarrhalis*, treatment, voice disorder.

INTRODUCTION

It has previously been shown[1,2] that adults with acute laryngitis harbor *Moraxella (Branhamella) catarrhalis* and *Hemophilus influenzae* in the nasopharynx in 50% to 55% and 8% to 15%, respectively, of cases, while these organisms are seldom isolated in nasopharyngeal specimens in healthy individuals of the same age group. Moreover, in patients with common colds without hoarseness, the isolation rate of *M catarrhalis* did not differ from that of healthy persons.[1] In most cases of acute laryngitis the patients experience common cold symptoms before the vocal symptoms become the most pronounced complaint. However, in our previous study,[1] viruses were not isolated from the nasopharynx in any of 40 patients, and only 2 displayed a significant titer rise against parainfluenza and influenza B viruses, respectively. In most patients *M catarrhalis* was spontaneously eliminated from the nasopharynx within 1 to 2 weeks.[2] These findings suggest that *M catarrhalis* in particular is in some way involved in the pathogenesis of acute laryngitis in adults.

Dysphonia and aphonia are the main reasons for seeking medical care for this condition. We found phenoxymethyl penicillin to have no effect either on the eradication of *M catarrhalis* and *H influenzae* from the nasopharynx, or on clinical signs and symptoms.[2] The lack of effect was not ascribable to β-lactamase production among the bacterial strains.

In the present double-blind, placebo-controlled study, the bacteriologic and clinical efficacy of erythromycin was evaluated, as this drug is highly active against *M catarrhalis* in vitro and effective in eradicating the organism from the nasopharynx.[3,4] Some preliminary results were previously reported.[5]

PATIENTS AND METHODS

One hundred six consecutive, otherwise healthy adults (all over 18 years of age) with acute laryngitis were examined. The inclusion criteria for acute laryngitis were hoarseness and a history of upper respiratory tract infection lasting no longer than 3 weeks, accompanied by inflammatory signs in the laryngeal, pharyngeal, and nasal mucosa (ie, increased vascular injection, purulent secretion, edema, or any combination of these signs). The exclusion criteria were pregnancy, chronic disease, allergy or intolerance to erythromycin, and any antibiotic therapy within the preceding 2 weeks. After otolaryngologic examination, erythromycin ethylsuccinate (Abboticin, Abbott, 0.5 g taken orally twice daily for 5 days) or placebo in identical tablets was administered according to a double-blind design. Six patients failed to keep scheduled appointments, and 1 presented with exanthema on the second day of treatment. Hence, the background data and findings at presentation were ana-

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TABLE 1. CHARACTERISTICS OF 106 PATIENTS WITH ACUTE LARYNGITIS

	Erythromycin (n = 53)	*Placebo (n = 53)*
Mean age	33.6	38.3
Male	15	10
Female	38	43
Smoker	10	14
Voice demand	41	46
Voice abuse	4	4
Previous laryngitis	32	28

Data are numbers of patients.

lyzed in 106 patients, and the data on treatment effect were analyzed in the 99 patients who completed the double-blind trial.

At presentation, historical data were recorded on a standard form. Otolaryngologic and phoniatric examinations were performed three times: at presentation and after 1 and 2 weeks. At all three visits specimens for bacterial culture were taken from the nasopharynx and cultured as previously described.[2] Signs of laryngitis, pharyngitis, and rhinitis were sought by mirror endoscopy or direct inspection and recorded on standard forms. Standardized voice recordings were obtained at all visits, and the patient's convalescent ("normal") voice was recorded 2 to 6 months later in 84 cases. The voice samples (ie, four per patient) were rated in randomized order by a listener group comprising four experienced clinicians. Scores (0 for normal voice and 1 for slight, 2 for moderate, and 3 for severe aberration) for 12 different voice qualities (rough voice, diplophonia, breathiness, vocal fry, episodes of aphonia, register abnormalities, register breaks, sonority, hyperfunction or hypofunction, and high or low pitch) were given by each listener for each recording.[6] The mean of the four listener scores for each individual voice sample was used for statistical evaluation. The Kendall coefficients of concordance between listeners for the voice qualities ranged from 0.45 to 0.91 in 30 randomly selected voice samples recorded at presentation.

TABLE 2. SYMPTOMS AND SIGNS AT PRESENTATION IN 106 PATIENTS WITH ACUTE LARYNGITIS

	Erythromycin (n = 53)	*Placebo (n = 53)*
History of upper respiratory tract infection	22	16
Abnormal findings		
Laryngitis*	51	52
Rhinitis	13	14
Pharyngitis	37	42
Mean subjective voice score	2.60	2.56
Bacterial pathogen isolated from nasopharynx	48	29

With exception of voice score, data are numbers of patients.

*Three patients not accessible for mirror laryngoscopy.

TABLE 3. ISOLATION OF RESPIRATORY TRACT PATHOGENS FROM NASOPHARYNX IN 99 PATIENTS WITH ACUTE LARYNGITIS TREATED WITH ERYTHROMYCIN (N = 48) OR PLACEBO (N = 51)

	Presentation		*After 1 wk*		*After 2 wk*	
Pathogen	*E*	*P*	*E*	*P*	*E*	*P*
*Moraxella catarrhalis**	30	19	5	13	9	7
Hemophilus influenzae	11	7	9	7	6	9
Streptococcus pneumoniae	1	3	0	2	0	1

Data are numbers of patients.

E — erythromycin, P — placebo.

*Elimination rate after 1 week was significantly higher in erythromycin group ($p \le .00038$, Fisher's exact test).

During the 2 weeks following presentation, each patient kept a daily record of the severity of voice symptoms, cough, sore throat, and rhinitis, rated thus: 0 for no symptoms and 1 for slight, 2 for moderate, and 3 for severe symptoms.

Nonparametric tests were used for statistical analysis, Fisher's exact probability test was used to compare the erythromycin and placebo groups for bacterial elimination rates, and the Mann-Whitney U test with adjusted z was used for all other comparisons. A probability level of 5% was considered significant. The investigation started in June 1986 and was concluded in June 1988.

RESULTS

Background Variables. Most (70%) of the patients were women, and 73% were nonsmokers. A large

TABLE 4. EFFECT OF ERYTHROMYCIN ON CLINICAL FINDINGS IN 99 PATIENTS WITH ACUTE LARYNGITIS

	Erythromycin (n = 48)	*Placebo (n = 51)*
Laryngitis		
Presentation	47*	51
After 1 wk	27	26
After 2 wk	13	17
Rhinitis		
Presentation	12	14
After 1 wk	4	4
After 2 wk	0	1
Pharyngitis		
Presentation	36	44
After 1 wk	23	30
After 2 wk	14	23

Data are numbers of patients.

*One patient not accessible for examination.

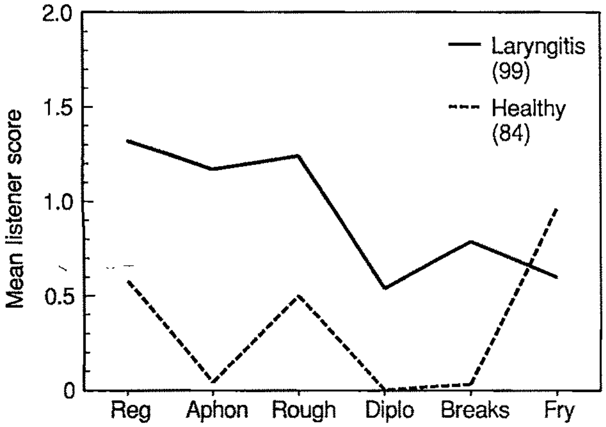

Fig 1. Voice profiles of all patients at presentation (laryngitis) and 2 to 6 months later (healthy). Reg — register abnormalities, Aphon — episodes of aphonia, Rough — rough voice, Diplo — diplophonia, Breaks — register breaks, Fry — vocal fry.

proportion reported voice function to be essential to their professional or social activities. There were no significant differences in background data between the placebo and the erythromycin groups (Table 1), nor did the groups differ with regard to history of preceding upper respiratory tract infection, findings at presentation, or subjective voice scores (Table 2).

Bacteriological Findings. The recovery rates of nasopharyngeal pathogens in the 106 patients initially included were *M catarrhalis* 50%, *H influenzae* 20%, and *Streptococcus pneumoniae*, 5%. Few of the patients harbored more than one of the pathogens. In Table 3, the isolation rates are given for the 99 patients who completed the study. Isolation of *M catarrhalis* at presentation was significantly more frequent in the erythromycin group than in the placebo group ($p \leq .012$, Fisher's exact test). After 1 week *M catarrhalis* was eliminated in 25 of the 30 patients in the erythromycin group, as compared to only 6 of 19 patients in the placebo group ($p \leq .00038$, Fisher's exact test). There was no difference between the two groups in the recovery rate of *M catarrhalis* after 2 weeks. The isolation rate of *H influenzae* was unaffected by erythromycin.

Clinical Evaluation. The clinical examinations after 1 and 2 weeks did not reveal any significant differences in the resolution of laryngitis, rhinitis, or pharyngitis between the erythromycin and placebo groups (Table 4).

Analysis of voice profiles of all patients, based on the mean scores of six selected voice qualities, showed that mean scores for all qualities except vocal fry were higher at presentation than the corresponding mean "healthy" scores 2 to 6 months later (Fig 1). Comparing the erythromycin and placebo groups on the various occasions, ratings of voice recordings did not reveal any statistical differences (Fig 2). After 1 week, the mean scores were markedly reduced, and the voice profiles appeared to be essentially normalized in both groups.

Patients' Evaluation of Symptoms. The scores for voice disturbance, sore throat, cough, and rhinorrhea are given in Table 5. At 1 week, significantly fewer voice complaints were reported by the erythromycin group than by the placebo group, and complaints of sore throat also tended to be fewer in the erythromycin group. At 2 weeks, significantly fewer cough complaints were recorded by the erythromycin group than the placebo group.

DISCUSSION

Moraxella catarrhalis is recognized as a pathogen occurring in both children and adults, causing otitis media,[7,8] sinusitis,[9] bronchitis,[10] and bronchopneumonia.[11-13] Like other respiratory tract pathogens, *M catarrhalis* is frequently isolated from the nasopharynx in small children,[14] but seldom in healthy adults. Thus, *M catarrhalis* was not isolated in any of 40 patients admitted to esophagoscopy (mean age 50 years) and matched week by week with the laryngitis patients.[1] Other investigators have confirmed that carriage of *M catarrhalis* in healthy adults is an uncommon feature: 2.4% and 1.1% in 123 and 565 healthy adults, respectively.[15,16] The high nasopharyngeal isolation rate of *M catarrhalis* reported here in adults with acute laryngitis was consistent with our previous results[1,2] and thus provided further evidence of *M catarrhalis* involvement in the pathogenesis of acute laryngitis in adults. *Hemophilus influenzae* may possibly be the second most important organism in this context. Whether other organisms, such as *Chlamydia pneumoniae*, may account for any symptoms is not known. As symptoms of laryngitis usually appear up to 14 days after the initial symptoms of a common cold, attempts to isolate viruses do not seem fruitful.[1]

The possible role of *M catarrhalis* in laryngitis is not defined so far. However, it has been reported that nasopharyngeal colonization by *M catarrhalis* is associated with pertussiform cough in children,[10] indicating a potential for distant involvement of the lower respiratory tract mucosa. In order to ascertain the microbial cause in laryngitis, demonstration of any agent in the larynx might be desirable. Unfortunately, such sampling, requiring direct laryngoscopy or translaryngeal aspiration, is not ethically justifiable. There is currently no established serologic method for *M catarrhalis* infection; furthermore, repeated exposure to this organism and the noninvasive nature of laryngitis imply that a serologic response

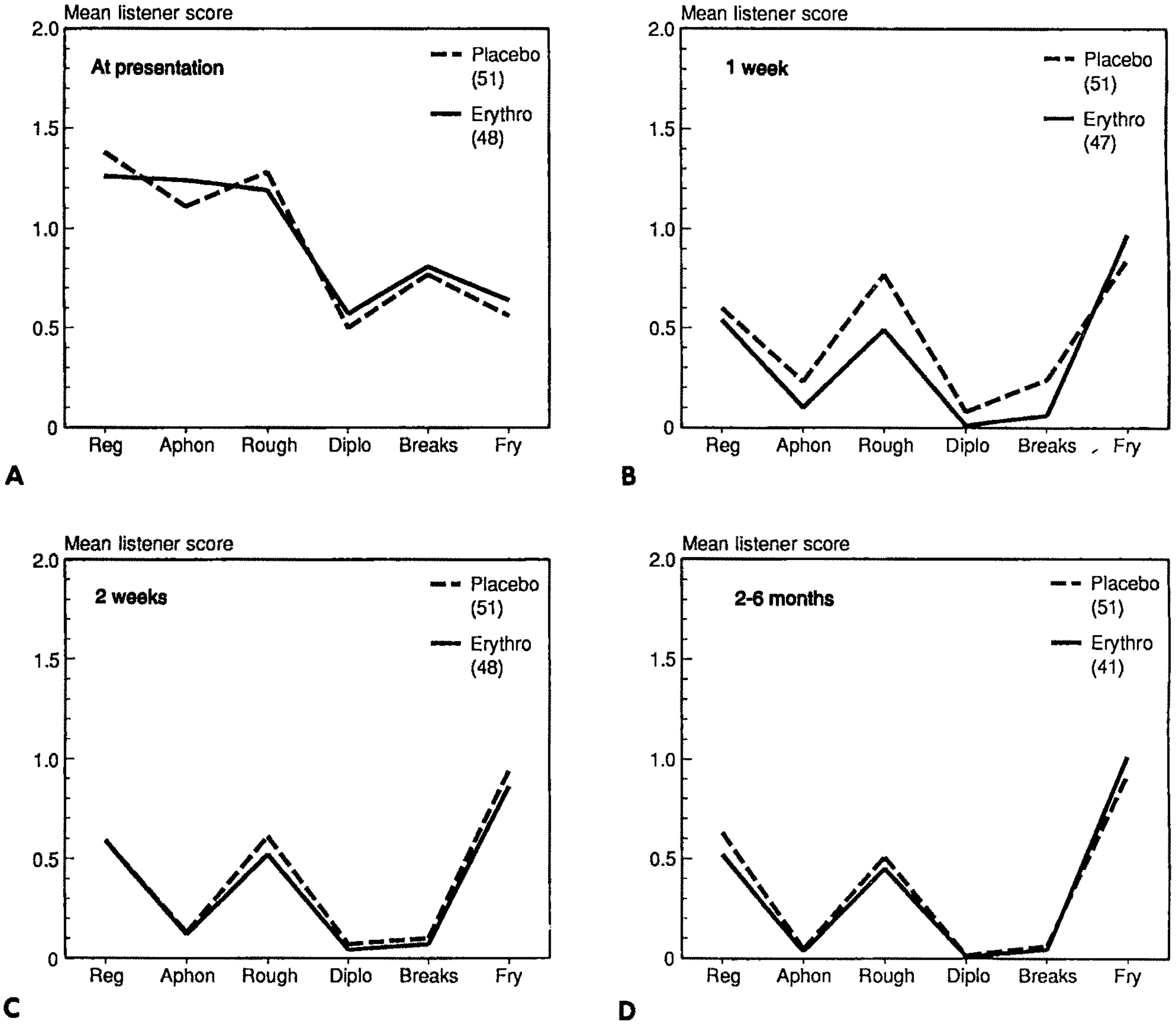

Fig 2. Voice profiles on various occasions in patients treated with erythromycin or placebo. Abbreviations as in Fig 1. A) At presentation. B) At 1 week. C) At 2 weeks. D) At 2 to 6 months.

may not be expected.

When the present investigation was performed, from June 1986 to June 1988, all *M catarrhalis* organisms isolated in our laboratory were susceptible to erythromycin (minimum inhibitory concentration [MIC] ≤ 0.5 mg/L). Although some (3%) erythromycin-resistant *M catarrhalis* organisms were isolated in Sweden in 1983,[17] it was not until 1991 that some (<1%) intermediately susceptible strains (MIC 1.0 to 4.0 mg/L) were recorded in our laboratory. In 1992 an increase was found, with 2.7% of isolates resistant (MIC > 4 mg/L) and 4.5% of strains intermediately susceptible. These findings were in accordance with recent reports of increasing emergence of erythromycin-resistant *M catarrhalis*.[18] The high elimination rate of *M catarrhalis*, but not of *H influenzae*, in the erythromycin-treated patients could be related to the fact that *M catarrhalis* is approximately eight times more susceptible to erythromycin than is *H influenzae*.[17]

Although erythromycin in an oral dose of 0.5 g twice daily for 5 days enhanced the elimination of *M catarrhalis* from the nasopharynx, neither objective evaluation of signs of upper respiratory tract infection nor the listener ratings of voice showed any effect of the drug. However, as compared to the controls, the erythromycin-treated patients experienced significantly less vocal disturbance after 1 week and less coughing after 2 weeks.

There may be several reasons for our difficulty to "objectively" show any effect of erythromycin. Endoscopic evaluation of the vocal tract may not be sensitive enough. Similar reasoning may apply to perceptual voice ratings. By this method, often used for clinical purposes, various voice qualities are rated

TABLE 5. SUBJECTIVE SYMPTOMS IN 99 PATIENTS WITH ACUTE LARYNGITIS TREATED WITH ERYTHROMYCIN OR PLACEBO

	Scores for Erythromycin Group (N = 48)				*Scores for Placebo Group (N =51)*				
	0	*1*	*2*	*3*	*0*	*1*	*2*	*3*	*p**
Voice disturbance									
Presentation	2	1	17	28	2	1	20	28	NS
After 1 wk	25	17	6	0	13	24	11	3	.0042
After 2 wk	39	9	0	0	34	13	4	0	NS
Sore throat									
Presentation	14	13	18	3	8	21	14	8	NS
After 1 wk	36	10	2	0	32	10	8	1	NS
After 2 wk	43	4	1	0	41	6	4	0	NS
Rhinorrhea									
Presentation	16	16	13	3	9	18	17	7	NS
After 1 wk	25	16	7	0	21	21	9	0	NS
After 2 wk	38	9	1	0	34	13	3	1	NS
Cough									
Presentation	15	17	11	5	11	16	18	6	NS
After 1 wk	28	12	8	0	22	15	12	2	NS
After 2 wk	43	4	1	0	37	10	4	0	.031

Scores: 0 — no disorder, 1 — slight disorder, 2 — moderate disorder, 3 — severe disorder.
NS — not significant.
*Mann-Whitney *U* test.

by a listener group; the results will thus be dependent on agreement between the listeners and their perceptual strategies.[19] However, in the present study, there was a satisfactory agreement between listeners, as indicated by high correlation coefficients for most voice qualities analyzed.

Although the present findings suggested that erythromycin may be useful in the treatment of adults with acute laryngitis, the general use of the drug for this disorder would not seem to be warranted. The disorder is self-limiting in most cases,[20] as was found among our placebo-treated patients. In addition, the differential diagnosis of acute laryngitis may be difficult, and most patients with acute voice disturbances are primarily considered as suffering from acute laryngitis.[21] Thus, because of diagnostic difficulties, patients with, for example, psychogenic dysphonia often receive antibiotics routinely and repeatedly.[22,23] Erythromycin treatment may, however, be justified for the purpose of rapid voice improvement in persons whose professional careers might be disturbed by even brief episodes of dysphonia.

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FUNCTIONAL SINUS TECHNIQUES

An international course on functional sinus techniques will be held in Graz, Austria, September 2-4, 1993. It will be conducted in English. For further information, contact Prof Dr Heinz Stammberger, University ENT-Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; telephone 43 316 385 3448; fax 43 316 385 3425.

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INTERNATIONAL CONFERENCE & COURSE ON STEREOTACTIC RADIOTHERAPY/RADIOSURGERY

The International Conference & Course on Stereotactic Radiotherapy/Radiosurgery will be held May 6-8, 1993, at Free University Hospital, Amsterdam, the Netherlands. For further information, contact Congress Secretariat (Ms A. Sol, Ms G. Luysterburg), VU-Ziekenhuis, Postbus 7057, 1007 MB Amsterdam, the Netherlands; phone 31-20-548 6163 or 548 6164; fax 31-20-548 6160 or 548 6101.

Ann Otol Rhinol Laryngol 102:1993

ANGIOGENESIS INDUCED BY HEAD AND NECK SQUAMOUS CELL CARCINOMA XENOGRAFTS IN THE CHICK EMBRYO CHORIOALLANTOIC MEMBRANE MODEL

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The angiogenic potential of head and neck tumors compared to nonneoplastic control tissues was investigated by using the chick embryo chorioallantoic membrane (CAM) as a bioassay for angiogenesis. Eighty tumor specimens from 10 patients with squamous cell carcinoma of the head and neck were grafted onto the CAM of 7-day chick embryos. The presence of tumor in the original explant was confirmed histologically. Forty-four embryos (55%) survived and were evaluated histologically at day 17. Tumors were growing on or invading the CAM mesoderm in 30 of 44 embryos (68%). Before harvesting of the membranes, the tumors and surrounding blood vessels were photographed, and the angiogenic responses were graded by a panel of blinded observers. Tumor explants elicited a significantly greater angiogenic response than nontumor controls ($p = .01$). We conclude that head and neck squamous cell carcinomas can induce an angiogenic response in vivo, presumably secondary to the production of an unidentified angiogenic factor, and that the chick embryo CAM is an effective model for quantifying angiogenesis induced by head and neck tumors.

KEY WORDS — angiogenesis, animal model, chick embryo chorioallantoic membrane, squamous cell carcinoma.

BACKGROUND

Angiogenesis or neovascularization is the formation of new blood vessels, which involves the proliferation, migration, and maturation of endothelial cells.[1] Angiogenesis occurs in normal and pathologic tissues, such as in the cyclical changes in the uterine endometrium, inflammation and wound healing, diabetic retinopathy, and tumor growth.[2] Tumor size and metastasis depend on the degree to which the tumor can stimulate an angiogenic response in the host. Previous experiments have shown that if tumor angiogenesis is limited or prevented, tumor growth is reduced.[3,4]

Soluble angiogenic factors were first isolated from tumors in the early 1970s,[5] and from the conditioned media of transformed cells in 1976.[6] Currently, there are several tumor- and tissue-derived angiogenic factors that have been purified.[7,8] These factors have been isolated from normal bovine and human tissues, human adenocarcinomas, and Walker rat 256 carcinoma.[7,9]

The ability of head and neck squamous cell carcinoma to stimulate neovascularization has not been directly studied. This paper reports the successful application of the chick chorioallantoic membrane (CAM) as a short-term in vivo system for culturing head and neck squamous cell carcinomas. We also demonstrate the ability of these tumors to stimulate an angiogenic response greater than that of nonneoplastic tissues.

METHODS

CAM Explants. Fertilized chicken eggs were incubated blunt-pole-up in saturation humidity at 39°C. Eggs were windowed on the fifth day of incubation and staged according to the criteria of Hamburger and Hamilton.[10] The bilayered shell membrane was removed to expose the epithelium of the vascularized CAM. The eggs were resealed with cellophane tape and returned to the incubator for 48 hours.

Tumor Preparations. Biopsy specimens of the upper aerodigestive tract were obtained at the time of extirpative surgery and histologically confirmed as squamous cell carcinoma. Tumor fragments were cut into approximately 100-mg pieces and incubated for 90 minutes at 37°C in room air in minimal essential medium (Sigma Co, St Louis, Mo) supplemented with gentamicin 50 µg/mL. Several tumor fragments from each patient were immediately fixed in 10% neutral buffered formalin and processed for light microscopy to confirm the presence of viable tumor in the explant. Control human tissues (muscle or skin) were obtained from regions of the surgical specimen uninvolved with tumor. Additional control tissues (brain, otocyst, muscle) were harvested from chick embryos. All tissues were processed in a fashion identical to that of the tumor explants prior to explan-

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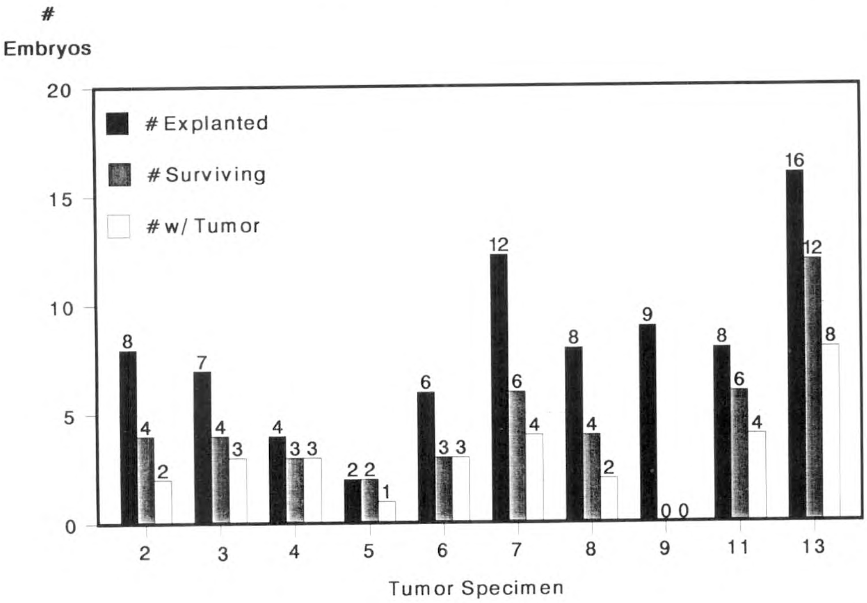

Fig 1. Survival of chorioallantoic membrane (CAM) head and neck tumor explants. Shows number of embryos receiving tumor explants, surviving, and containing viable tumor nodules. Of 44 embryos surviving at day 17, 30 (68%) had tumor growing on or invading CAM mesoderm.

tation.

Tissue fragments were explanted onto the vascularized CAM of 7-day chick embryos after gentle trauma to remove the CAM epithelium and facilitate graft survival.[11,12] Grafts were placed on the CAM near the Y branch of a large blood vessel.

At day 17 the tissue explants and surrounding CAM were photographed for later analysis. Membranes and explants were then fixed in 10% neutral buffered formalin, processed for standard light microscopy, and embedded in paraffin. Sections (8 μm) were cut on a standard rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin. Embryos were counted as positive for tumor growth if viable tumor growing on the CAM mesenchyme was demonstrated histologically.

Determination of Angiogenesis Scores. Angiogenesis was quantified according to the method of Vu et al.[13] Six blinded observers evaluated projected slides of 10 nontumor tissues. These control tissues included human skin or muscle obtained from the surgical specimen or embryonic chick muscle, brain, or otocyst. Twenty-five slides of tumor explants were also projected at the same magnification. Each observer graded each projected slide on the following 0 to 4 scale based on the angiogenic response of the CAM and the amount of new capillary growth. Angiogenesis scores were determined as follows: 0 indicated no significant increased angiogenesis over the background of the CAM, 1 indicated some hyperemia with increased vessel tortuosity, 2 indicated longer vessels oriented directionally toward the tissue explant, 3 indicated significantly more vessels per unit area with radial orientation around the explant, and 4, given to only two explants, indicated a dramatic angiogenic response with significant neovessel ingrowth and hyperemia. The median score for each specimen was determined.

Statistical Analysis. A Kruskal-Wallis one-way analysis of variance was performed to measure differences in median angiogenesis scores between observers. The median angiogenesis scores of control and tumor tissues were compared by means of the Mann-Whitney U (rank sum) test, where $p < .05$ is considered significant. A Friedman's two-way analysis of variance was performed to test differences between the median angiogenesis scores of tumor tissues, controlling for the effects of individual observers.

RESULTS

Eighty tumor explants from 10 patients with squamous cell carcinomas of the head and neck were performed. Forty-four (55%) of the embryos survived and were evaluated at embryonic day 17. Of these, viable tumor explants were identified in 30 (68%; Fig 1). Adequate photomicrographs of tumor explants for determination of angiogenesis scores were available in 25 of these 30 explants, and were subsequently used for statistical analysis.

All tumor explants demonstrated the characteristic histopathologic features of squamous cell carcinoma. Tumor nodules showed cellular pleomorphism with intracellular bridges, nuclear atypia, and mitoses (Fig 2). There was evidence of keratinization and the formation of keratin pearls. Tumor explants demonstrated "pushing" borders that infiltrated into the mesodermal layers of the CAM (Fig 3). The histologic appearance of the tumor explants closely re-

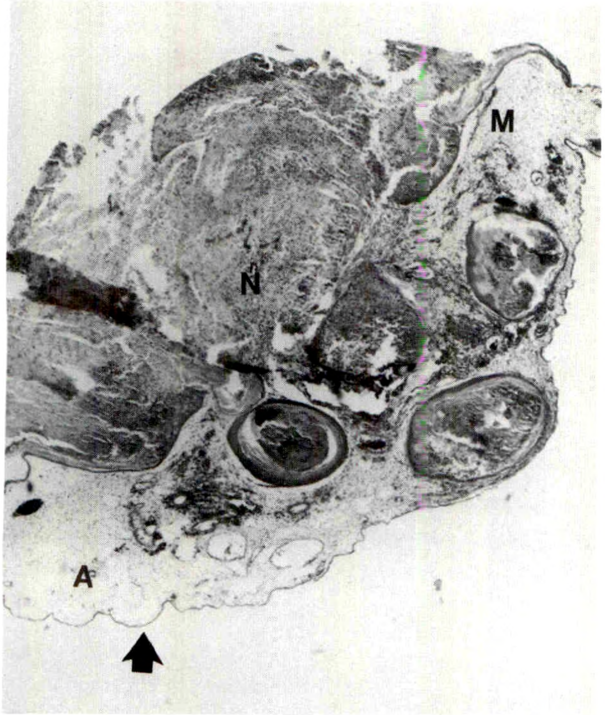

Fig 2. Low-power light micrograph of nodule (N) of head and neck squamous cell carcinoma explanted onto CAM (A; H & E, original x4). Arrow indicates mesodermal side of CAM facing yolk sac. Note relatively acellular CAM mesoderm (M) adjacent to tumor nodule.

sembled that of the original tumor.

Interobserver variability was assessed with a Kruskal-Wallis one-way analysis of variance. The difference in median angiogenesis scores between observers was found to be nonsignificant (p = .77), demonstrating close agreement between observers.

When compared to nontumor control tissues, squamous cell carcinoma xenografts stimulated an augmented angiogenic response (Fig 4). The median angiogenesis score for the squamous cell carcinoma explants was 2.30 (SD ± 0.94); that for control (nontumor) tissue was 1.25 (SD ± 0.84). The Mann-Whitney rank sum test was used to determine statistical significance at a p = .01 level (Fig 5). Tumor nodules were characterized by an increased radial outgrowth of CAM vessels in a "spoke wheel" pattern around the explant periphery. It appeared that more vessels per unit area were seen on tumor explants than on controls. Control explants did not stimulate the directional pattern of neovascularization observed in tumors. There were no differences in the angiogenic response of the CAM between control tissue types. Survival rates of tumor explants did not appear to be related to median angiogenesis scores of individual tumors. Controlling for the effect of individual observers, we found a significant variance in angiogenesis scores (p = .0001; Friedman's two-way analysis of variance), suggesting that significant differences in angiogenic response exist among squamous cell carcinomas.

Since multiple tumor explants were obtained from individual patients, there existed a potential bias in determination of a mean angiogenesis score for all tumors. Therefore, the median values of pooled samples (nine patients) were compared. A significant increase in angiogenesis was still apparent in tumor compared to nontumor explants (p = .04).

DISCUSSION

Tumor size and metastatic potential depend on the degree to which a tumor can stimulate an angiogenic response in the host. Tumor size is limited to 1 mm^3

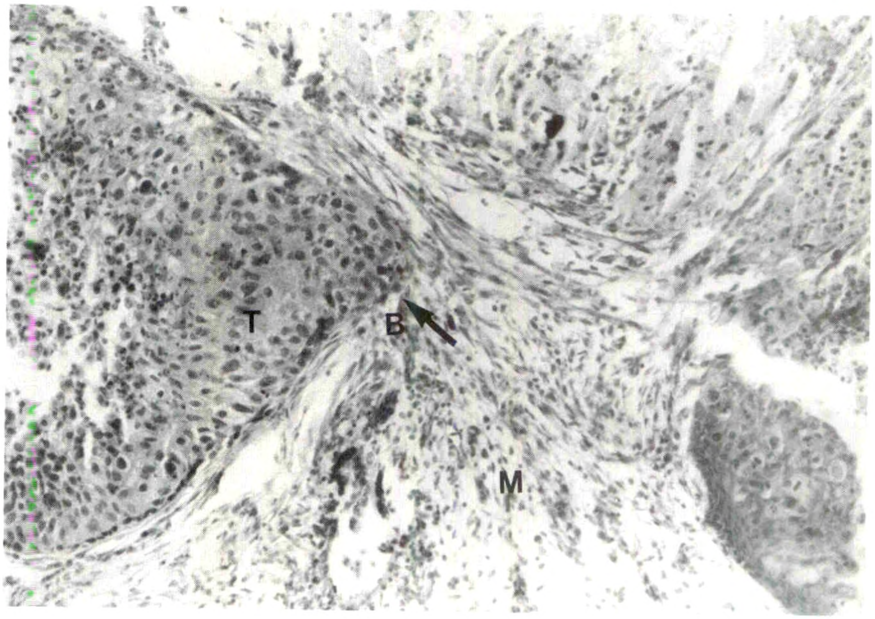

Fig 3. High-power micrograph of head and neck squamous cell carcinoma explanted onto CAM (H & E, original x20). Note pushing border (B) as tumor (T) infiltrates CAM mesoderm (M). Arrow — tumor-CAM interface.

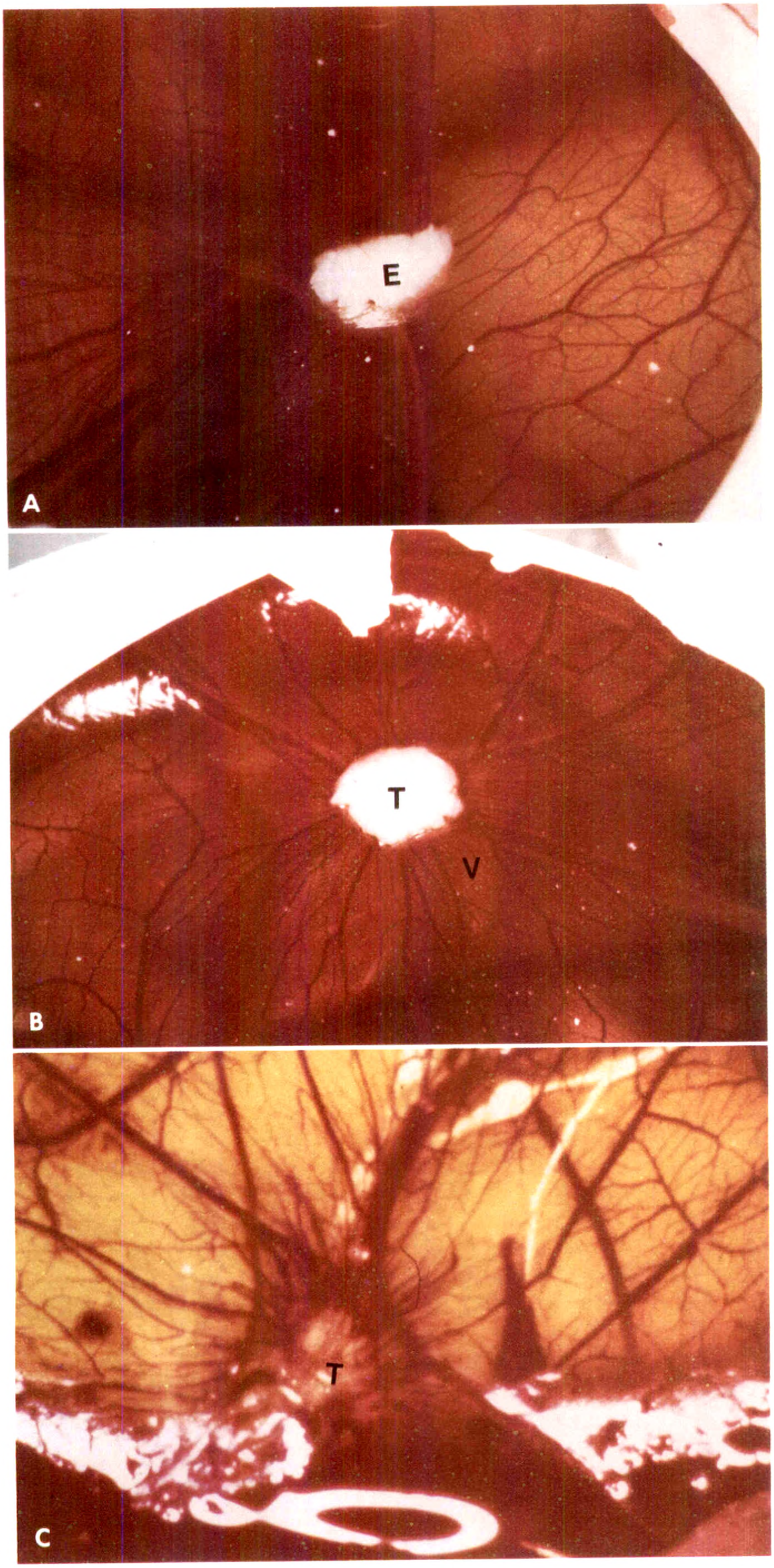

Fig 4. Explants. A) Control (nontumor) explant (E) at day 17 on CAM. Note absence of significant angiogenic response and lack of blood vessels radiating around tissue. B) Explant of tumor (T) at day 17. Note presence of increased number and density of blood vessels (V) compared to A. Also note radial outgrowth ("spoke wheel") pattern. C) Dramatic example of increased CAM neovascularization induced by tumor (T). Number of vessels per unit area is increased significantly compared to A and B. This specimen was scored 4 (see text) by all observers.

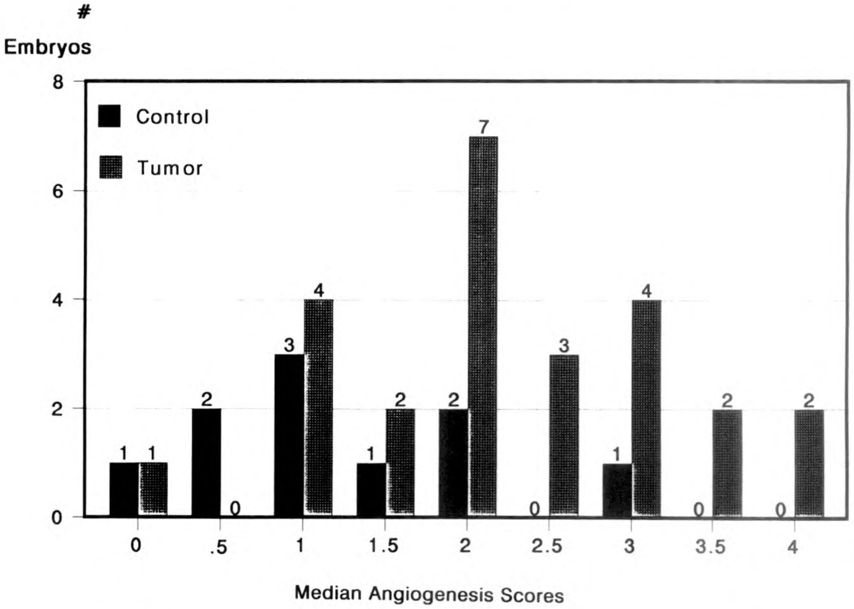

Fig 5. Distribution of control and tumor explant angiogenesis scores. Mann-Whitney rank sum analysis of median angiogenesis scores demonstrated significantly increased neovascularization induced by tumor explants (p = .01).

by the diffusion of nutrients and metabolic by-products into or out of an enlarging tumor mass. Failure to establish adequate tumor neovascularization results in necrosis.[1,3,14] Patients with head and neck squamous cell carcinomas often present with tumors several centimeters in greatest dimension. These tumors, therefore, must be capable of initiating an angiogenic response. We have successfully demonstrated the angiogenic properties of head and neck squamous cell carcinomas with an in vivo bioassay.

Angiogenic factors have been isolated from both virally transformed and spontaneous solid tumors. The 14 kd polypeptide angiogenin has been isolated from human colon adenocarcinoma, lung carcinoma, hepatoma, and the Walker rat carcinoma.[15] Other tumors have been shown to induce angiogenesis without the isolation of specific angiogenic molecules. However, angiogenesis or angiogenic factors derived from squamous cell carcinoma of the head and neck have not been reported.

Angiogenesis is a process that involves interactions among endothelial cells, elements of the connective tissue matrix, and soluble factors.[7,8] It follows that more complex in vivo systems are necessary for studying the process as a whole. Several quantifiable models of angiogenesis have been described, including the rabbit cornea,[16] rat retroperitoneal sponge,[17] rat peritoneum,[9] and chick embryo CAM.[11,12] The CAM is a simple, inexpensive, and quantifiable[13] alternative to other in vivo techniques and continues to be used extensively in a variety of applications.[18-21]

Several in vivo models have been used to study various aspects of the biology of head and neck squamous cell carcinoma xenografts. These include the hamster cheek pouch, guinea pig eye, and rodent brain.[22] Tumor growth, metastasis, and chemosensitivity have been studied primarily in congenitally (nude) or pharmacologically immunosuppressed mice. Xenografts have been established by implantation into the flank or chest,[23,24] injection into the floor of the mouth,[25] or injection into the subrenal capsule.[26] However, the angiogenic capacity of these tumors has not been studied.

Lauk et al[27] have studied the microvascular anatomy and spatial distribution of proliferating capillaries in head and neck squamous cell carcinoma explants in nude mice. Using histomorphometric techniques, they demonstrated that the vascularity of these tumors is altered after explantation into mice, but did not comment on the degree to which tumors stimulated neovascularization in the host.[27] As in our study, the histopathologic characteristics of their original tumors were retained in the explants. Similar observations were made by Tilgen et al[28] studying morphological and ultrastructural characteristics of skin squamous cell carcinomas in vitro and as xenografts.

Previous studies of CAM explants of head and neck squamous cell carcinoma have been limited. Ossowski and Reich[29] and Ossowski[30] have used the CAM extensively to study the relationships between plasminogen activators and metastases using the HEp-3 (human buccal squamous cell carcinoma) cell line. However, these studies did not examine the angiogenic capacity of this tumor, or correlate angiogenesis with invasiveness or metastatic potential.

The ability to induce new blood vessel growth in the host is not unique to head and neck squamous cell carcinoma. Both solid and epithelial tumor explants and aliquots from existing cell lines[9,12,18] have been shown to stimulate some angiogenic response in the chick embryo CAM. However, our study is the first to specifically investigate the in vivo angiogenic properties of a series of epidermoid neoplasms of the head and neck.

The production of angiogenesis factors by squamous cell carcinomas of the head and neck may play a role in the growth and metastasis of these tumors. Identification of such factors may allow the development of new treatment strategies that utilize antagonists to these factors. Several investigators have described inhibition of tumor-induced angiogenesis in experimental systems, including the CAM.[31,32] It is possible that the effect of certain biologic response modifiers (eg, tumor necrosis factor or interferon) on tumor growth may be mediated by inhibition of angiogenic factors.[33]

Currently, we are attempting to isolate the angiogenic fractions of head and neck squamous cell carcinoma extracts so that we can determine whether the angiogenesis induced by these tumors is due to one of the known angiogenic peptides or to a novel polypeptide.

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20TH INTERNATIONAL CONFERENCE ON COCHLEAR IMPLANTS

The 20th International Conference on Cochlear Implants will be held June 23-25, 1993, at the Ritz-Carlton Hotel, San Francisco, California. For further information, contact University of California, Extended Programs in Medical Education, Room LS-105, San Francisco, CA 94143-0742; (415) 476-4251.

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FOURTH INTERNATIONAL SYMPOSIUM AND WORKSHOPS ON INNER EAR MEDICINE AND SURGERY

The Fourth International Symposium and Workshops on Inner Ear Medicine and Surgery will be held July 17-24, 1994, in Snowmass-Aspen, Colorado. For more information, please contact I. Kaufman Arenberg, MD, Executive Director and Program Chairman, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; phone 303-788-4320 or 303-850-9545; fax 303-788-4234.

Ann Otol Rhinol Laryngol 102:1993

EFFICACY OF CEFTIBUTEN FOR ACUTE OTITIS MEDIA CAUSED BY *HEMOPHILUS INFLUENZAE:* AN ANIMAL STUDY

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RAMAT-HASHARON, ISRAEL

Ceftibuten is a new oral cephalosporin with an unusual stability to β-lactamases that can hydrolyze other extended-spectrum cephalosporins. Using the chinchilla animal model, we compared the efficacy of ceftibuten ($n = 33$) with that of saline ($n = 34$), ampicillin ($n = 32$), and cefixime ($n = 31$) for the treatment of acute otitis media caused by β-lactamase–producing nontypeable *Hemophilus influenzae*. Ceftibuten was superior to ampicillin regarding the time necessary to sterilize the middle ear ($p < .001$) and eliminate effusion ($p < .001$). The mean days of therapy required for bacteriologic cure were 2.57 for ceftibuten, 2.95 for cefixime, 7.95 for ampicillin, and 8.16 for saline. At the conclusion of therapy, chinchillas treated with ceftibuten had a significantly lower prevalence of positive cultures and middle ear effusion than did animals treated with ampicillin. No significant differences were observed between ceftibuten and cefixime. The results of this randomized, investigator-blinded experiment warrant further consideration of ceftibuten as a second-line agent for acute otitis media caused by ampicillin-resistant *H influenzae*.

KEY WORDS — acute otitis media, ceftibuten, cephalosporins, drug therapy.

INTRODUCTION

Acute otitis media (AOM) is responsible for over 11 million physician encounters per year by children under 5 years of age in the United States.[1] *Streptococcus pneumoniae* and *Hemophilus influenzae* are the most frequent bacterial pathogens isolated from the middle ear of children with AOM.[2] Amoxicillin, the traditional first-line agent for otitis media, has excellent activity against *S pneumoniae*, but is ineffective against the 15% to 30% of nontypeable *H influenzae* isolates that produce β-lactamase. Consequently, a variety of second-line antimicrobials have been developed that are β-lactamase–resistant, but their use has been limited by problems of gastric intolerance, high cost, or limited coverage of gram-positive bacteria.

Oral cephalosporins interfere with bacterial cell wall synthesis in a manner identical to the penicillins, but provide enhanced activity against certain gram-negative organisms. Cefaclor is used widely for AOM, yet the middle ear effusion concentrations are well below the 90% minimum inhibitory concentration (MIC90) for many β-lactamase–producing *H influenzae* strains.[3] Cefuroxime axetil, an extended-spectrum cephalosporin with excellent *Hemophilus* coverage, has limited use in young children because of its bitter taste and the unavailability of a liquid preparation. At present, cefixime is the best-tolerated and most efficacious oral cephalosporin for AOM caused by ampicillin-resistant β-lactamase–producing nontypeable *H influenzae*.[4,5] Ceftibuten (SCH 39720) is a promising new drug with excellent bioavailability after oral administration, and an unusual stability to certain TEM and SHV β-lactamases that can hydrolyze other extended-spectrum cephalosporins.[6] Although the MIC of ceftibuten against β-lactamase–producing *H influenzae* is extremely low (<0.06 mg/L),[7] the in vivo efficacy of the drug for AOM is unknown.

The purpose of this investigation was to compare the in vivo efficacy of ceftibuten with that of cefixime, ampicillin, and a saline control for the treatment of AOM caused by *H influenzae*. We chose the chinchilla model of otitis media to permit complete control over the selection of bacterial pathogen. Middle ear infection was induced by inoculating the tympanic cavity with *H influenzae*, and drug efficacy was determined by using serial culture, tympanom-

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Presented in part at the Fifth International Symposium on Recent Advances in Otitis Media, Fort Lauderdale, Florida, May 22-24, 1991.

REPRINTS — Richard M. Rosenfeld, MD, MPH, Dept of Otolaryngology, Long Island College Hospital, 340 Henry St, Brooklyn, NY 11201.

SUMMARY OF OUTCOME MEASURES AND STATISTICAL TESTS

	Treatment Group				*p Value*			
Outcome Measure	*Saline (n = 34)*	*Ampicillin (n = 32)*	*Cefixime (n = 31)*	*Ceftibuten (n = 33)*	*All Groups Combined*[a]	*Cefixime vs Ampicillin*[b]	*Ceftibuten vs Ampicillin*[b]	*Ceftibuten vs Cefixime*[b]
Mean days surviving[c]	9.41	9.50	9.03	9.64	.392			
Serial culture right ear								
Sample size[d]	32	29	22	28				
Mean days until sterile[c]	8.16	7.95	2.95	2.57	.001*	.001*	.001*	.542
Serial tymp left ear								
Sample size[e]	18	21	20	22				
Mean days until normal[c]	8.59	7.90	7.55	6.24	.020*	.614	.016*	.066
Serial oto left ear								
Sample size[e]	21	22	26	27				
Mean days until normal[c]	7.79	7.18	6.36	5.36	.007*	.254	.014*	.155
Serial tymp and oto left ear								
Sample size[e]	24	25	28	29				
Mean days until normal[c,f]	8.50	7.87	6.87	6.23	.001*	.150	.004*	.133
Final status of left ear								
Sample size[g]	29	29	25	31				
% Positive culture[h]	26.0	24.1	0.0	0.0	.003*	.006*	.005*	
% Abnormal oto[h]	37.9	31.0	20.0	6.5	.015*	.535	.020	.223
% Abnormal tymp[h]	41.4	34.5	24.0	12.9	.064	.553	.068	.315
% Abnormal oto or tymp[h]	48.3	41.4	28.0	12.9	.013*	.230	.013*	.190

tymp — tympanometry, oto — otomicroscopy .

[a] $p < .05$ is significant (asterisk in table).

[b] $p < .017$ is significant (asterisk in table); Bonferroni adjustment for three comparisons with overall α .05.

[c] Kaplan-Meier life table analysis with Mantel-Cox comparison of survival curves.

[d] Excludes animals that were culture-negative for *H influenzae* at start of treatment.

[e] Excludes animals that never became abnormal during course of treatment.

[f] Days until *both* tympanometry and otomicroscopy became normal.

[g] Excludes animals not alive at completion of treatment.

[h] Likelihood ratio χ^2 for combined analysis; Fisher's exact test (two-tailed) for group comparisons.

etry, and otomicroscopy.

MATERIALS AND METHODS

Study Protocol. One hundred thirty healthy adult chinchillas (*Chinchilla laniger*), weighing an average of 450 g, were used. Before entry into the study, we observed all animals for at least 2 weeks and determined all ears to be free of effusion by tympanometric assessment. Animal procedures were approved by the Children's Hospital Animal Research Care Committee and performed under sedation with ketamine hydrochloride, 20 mg/kg of body weight.

On day 0, all chinchillas were inoculated bilaterally with 0.1 mL of a buffered saline solution containing a mean time-averaged concentration of 82 colony-forming units of nontypeable β-lactamase–producing *H influenzae*. The organism was the first passage of a clinical isolate obtained from a child with AOM; it was highly susceptible to ceftibuten and cefixime (MIC ≤ 0.06 mg/L) but resistant to ampicillin (MIC 32 mg/L). Since our goal was to assess ceftibuten efficacy for ampicillin-resistant *Hemophilus*, we included an ampicillin treatment group to permit meaningful analysis, even though the high MIC suggested that ampicillin might be no more effective than the saline controls. The inoculum was introduced into the tympanic cavity through a transbullar approach as previously described.[8]

We randomized the animals into four groups and examined them every other day after inoculation (days 4, 6, 8, 10, 12, and 14). Under an investigator-blinded protocol, animals received intramuscular saline solution (n = 34 animals), ampicillin 150 mg/kg (n = 32), cefixime 8 mg/kg (n = 31), or ceftibuten 30 mg/kg (n = 33) every 8 hours. The doses for ampicillin and cefixime were based on an earlier chinchilla study[4]; the ceftibuten dose was derived from pharmacokinetic tests performed on a separate group of animals. The 10-day course of treatment began on day 4 (4 days after inoculation) and continued through day 14.

On alternate days we examined all animals by means of bilateral tympanometry, otomicroscopy of the left ear, and culture of the right middle ear through

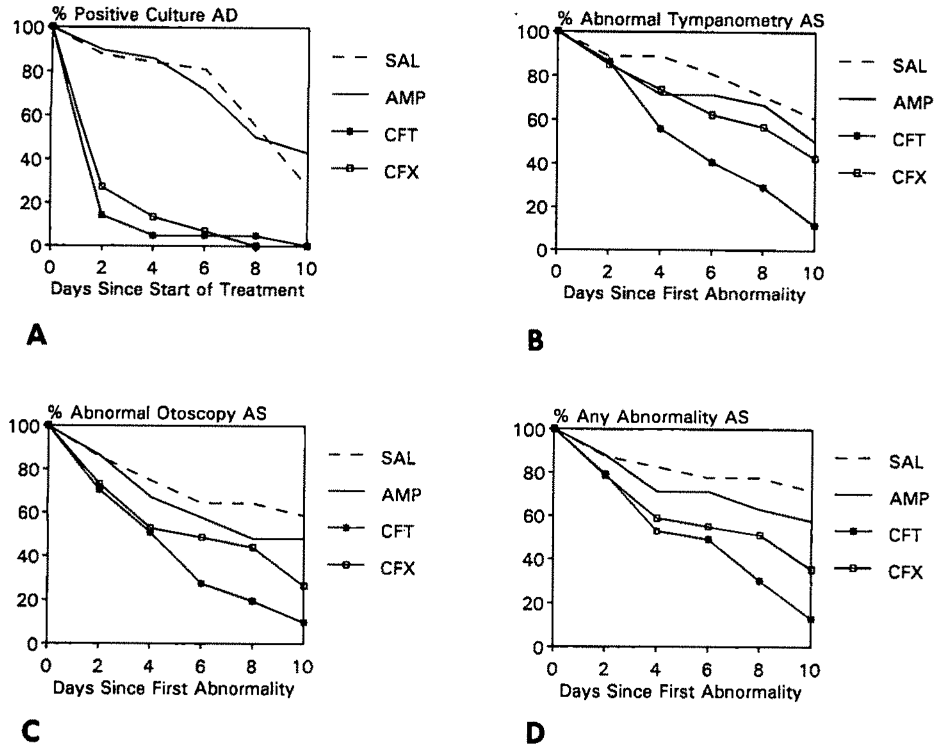

Fig 1. Line graphs approximating Kaplan-Meier curves for chinchillas receiving saline (SAL), ampicillin (AMP), ceftibuten (CFT), or cefixime (CFX). AS — left ear, AD — right ear. Diagrams depict results of A) serial culture, B) tympanometry, C) otomicroscopy, and D) tympanometry combined with otomicroscopy. Both cephalosporins performed better than ampicillin regarding serial culture; however, only ceftibuten was statistically better than ampicillin for other three outcome measures.

a transbullar approach. An automatic impedance meter (Teledyne model TA-7A) was used for tympanometry; results were considered abnormal if compliance was less than 0.6 mL or pressure was less than -60 mm H2O. Otomicroscopy was interpreted as abnormal if the tympanic membrane was opaque, inflamed, or bulging or if an air-fluid level was present. Cultures were considered positive when the inoculated organism was recoverable. The left middle ear was not cultured until day 14 so that the duration of the effusion could be documented without the confounding effects of the culturing process.

Statistical Analysis. All data were collected with standardized forms and carefully screened for errors and inconsistencies. Statistical tests were performed with BMDP software (1990 release) on a mainframe computer.[9]

The primary longitudinal outcome measure was days until sterilization of serial culture from the right middle ear; secondary longitudinal measures included mortality, and days until resolution of the left middle ear effusion (tympanometry and otomicroscopy). Kaplan-Meier survival analysis with Mantel-Cox comparison of survival curves was used to detect heterogeneity among the four treatment groups, as well as significant intergroup differences (cefixime versus ampicillin, ceftibuten versus ampicillin, and ceftibuten versus cefixime). For serial tympanometry and otomicroscopy, the number of days required to return to normal after first becoming abnormal was used in the survival analysis; animals that died with abnormalities, or remained abnormal at the conclusion of the study (day 12) were considered censored observations. To maintain an α level of .05 for the experiment as a whole, the Bonferroni adjustment for three a priori comparisons was used.

Point prevalence outcome measures related to the status of the left (nonviolated) ear at the completion of treatment. Separate analyses were performed for culture, otomicroscopy, and tympanometry results by using the likelihood ratio χ^2 and Fisher's exact test; a priori comparisons were performed with the α level adjusted as described above.

RESULTS

Although 16 chinchillas (12%) died prior to completion of the trial, survival analysis allowed us to incorporate observations up to the time of death. There were no significant differences in mean survival for animals in each of the four treatment groups (see Table). Both ceftibuten and cefixime resulted in more rapid sterilization of the right ear than ampicillin, but there were no significant differences between the two cephalosporins (see Table and Fig 1). All of the other longitudinal outcome measures — serial tympanometry, serial otoscopy, and combined analysis — demonstrated significant heterogeneity among the four groups; in addition, ceftibuten was significantly better than ampicillin for all intergroup comparisons. Although ceftibuten performed no better than ampicillin for each of these three outcome measures, there were again no significant differences

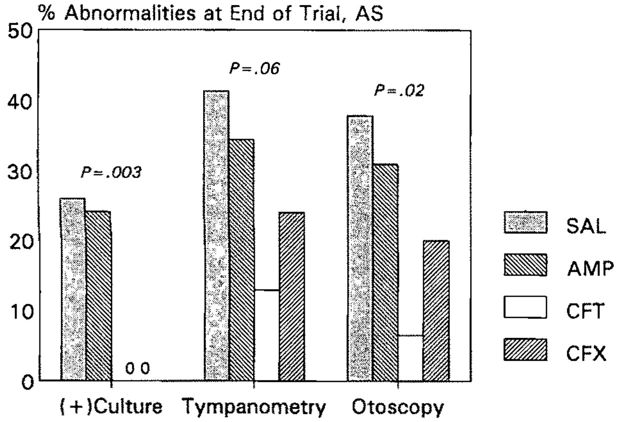

Fig 2. Prevalence of positive cultures and abnormalities of left, nonviolated ear (AS). Both cephalosporins performed better than ampicillin, regarding positive culture; similar, but not significant, trend was observed for other outcome measures. SAL — saline, AMP — ampicillin, CFT — ceftibuten, CFX — cefixime.

between the two cephalosporins.

The point prevalence outcomes based on the status of the left ear at completion of treatment are shown at the bottom of the Table and are illustrated in Fig 2. Using positive culture as the outcome, both cephalosporins were more effective than ampicillin in eradicating *H influenzae* from the middle ear. The prevalence of abnormal otoscopy varied significantly among the groups as a whole, but the superior results in the ceftibuten group failed to achieve significance during intergroup comparisons. There was no overall difference among groups when using abnormal tympanometry as the outcome. For combined tympanometry-otoscopy, ceftibuten was better than ampicillin, but cefixime was not. There were no significant differences between ceftibuten and cefixime for any of the prevalence measures.

DISCUSSION

Oral cephalosporins have found widespread use in the treatment of pediatric head and neck infections. They are more expensive than the oral penicillins, but offer the advantages of greater palatability and somewhat greater safety.[10] For the particular strain of ampicillin-resistant *H influenzae* that we studied, ceftibuten and cefixime were equally efficacious in their ability to rapidly achieve a bacteriologic cure of AOM. Concern has been raised, however, that extended-spectrum cephalosporins in general, and cefixime in particular, may have diminished activity against *S pneumoniae* when compared with the aminopenicillins.[5,11] Since pneumococcus is the leading cause of otitis media in children, we compared the efficacy of ceftibuten and cefixime with amoxicillin in another randomized chinchilla experiment.[12] Whereas the saline controls performed worse than all other groups ($p < .05$), no significant differences were detected among the three antibiotics regarding the time required to sterilize the middle ear, or the prevalence of positive cultures for *S pneumoniae* after 10 days of therapy.

The chinchilla animal model of AOM, first described by Giebink et al[13] in 1976, has proven to be a rapid and relatively inexpensive method for assessing drug efficacy in a large and homogeneous study population.[4] The model permits control of various study parameters not amenable to documentation in clinical investigations, such as the time to disease onset, time to treatment, degree of compliance with proposed therapies, and selection of bacterial pathogen. Limitations relate primarily to the route of drug administration and the virulence of the challenge organism. Since oral administration of antimicrobials in the chinchilla can result in serious gastrointestinal toxicity, suitable intramuscular doses must be substituted, based on pharmacokinetic studies. Chinchilla mortality in previous studies using *H influenzae* has ranged from 10% to 60%[8]; the 12% rate observed in this study correlates well with the 15% mortality reported by Hotaling et al[4] in an earlier investigation of cefixime efficacy.

Our experiment has shown that ceftibuten administered at 30 mg/kg of body weight every 8 hours is more effective than ampicillin for rapidly sterilizing the middle ear of chinchillas with AOM caused by β-lactamase–producing nontypeable *H influenzae*. No adverse side effects of the drug were noted. At the conclusion of therapy, chinchillas treated with ceftibuten had a significantly lower prevalence of positive cultures and middle ear effusion than did animals treated with ampicillin. No significant differences were detected between ceftibuten and cefixime for any outcome measure.

On the basis of our findings, we consider ceftibuten worthy of further consideration as a second-line antimicrobial agent for the treatment of AOM that has not responded satisfactorily to amoxicillin. Nonetheless, caution must be exercised when extrapolating the results of chinchilla trials to humans. We tested only a single strain of *H influenzae*, and administered the drug intramuscularly, not orally. Ceftibuten does, however, possess broad in vitro activity against β-lactamase–producing nontypeable *H influenzae*, as evidenced by an MIC of ≤0.06 mg/L for 17 (89%) of an additional 19 clinical isolates that we tested. Further research is needed to document in vivo efficacy for other strains of *Hemophilus*, and to determine if comparable benefits can be achieved after oral administration.

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ROUND WINDOW MEMBRANE IN SEROUS AND PURULENT OTITIS MEDIA

STRUCTURAL STUDY IN THE RAT

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The rat was used as an animal model to reveal structural alterations in the round window membrane (RWM) during serous otitis media (SOM) and purulent otitis media (POM) over a 6-week period. Comparison of POM animals and control animals showed that the RWM in the former became almost six times as thick as that in controls, whereas that of SOM animals was twice as thick. The structural changes in the RWM in POM animals were confined mainly to the epithelium facing the middle ear cavity and the subepithelial space, which was invaded by inflammatory cells and exhibited dilated vessels. The normal flat epithelium was transformed via cuboidal cells to a cylindric epithelium containing both ciliated and goblet cells. In SOM animals, light microscopy revealed only minor changes in the RWM structure. Ultrastructurally, however, the connective tissue layer exhibited dense aggregations of collagen, increased numbers of fibroblasts, and, in one case, elastic fibers. This last phenomenon was not observed in either POM ears or normal ears. The study showed that various inflammatory conditions of the middle ear, both noninfectious (SOM) and infectious (POM), can cause different structural alterations of the RWM. These structural changes may influence passage through the RWM differently.

KEY WORDS — light microscopy, purulent otitis media, round window membrane, serous otitis media, ultrastructure.

INTRODUCTION

Otitis media with effusion (OME) is an inflammation of the middle ear cavity (MEC) associated with an accumulation of effusion material — middle ear effusion (MEE). The effusion can be serous, purulent, mucoid, or a mixture of these different types of fluid.[1] Otitis media with effusion is the most common cause of hearing loss among infants and young children. In most cases it leads to a conductive hearing loss, and in some protracted cases it can interfere with speech and language development. The usual remedy for such hearing loss is myringotomy and intubation, which eliminates the fluid from the MEC. Occasionally, a sensorineural hearing loss occurs in connection with OME.[2-6] The round window membrane (RWM), hidden in the round window niche, is the thinnest and the main soft structure separating the middle ear from the inner ear. Consequently, it has been assumed that inner ear disturbances in OME are mediated through the RWM.

The rat has attracted increasing interest as an animal model suited to studies of inflammatory conditions of the middle ear. Several distinct types of effusion material can be provoked experimentally.[7-9] Previous studies in the rat have shown that different otitis media conditions elicit different changes in the epithelial lining of the middle ear mucosa.[10] In purulent otitis media (POM) the pars flaccida becomes thickened and the inner epithelial lining is replaced by a pseudostratified squamous epithelium containing ciliated and secretory cells, whereas in serous otitis media (SOM) the pars flaccida appeared fibrotic, with large dilated vessels but without any major alterations of the epithelium facing the MEC. A preliminary study revealed similar changes of the epithelium of the RWM in SOM and POM.[11] The purpose of the present investigation, in two otitis media models in the rat, was to elucidate the RWM structure in SOM (noninfectious) and POM (infectious) conditions[12] over a 6-week period. The study was performed by both light and electron microscopic techniques.

MATERIAL AND METHODS

Fifty male Sprague-Dawley rats weighing 300 to 360 g were used for the study. The animals were divided into two groups and subjected to one of the surgical procedures described below. For these procedures the animals were anesthetized with methohexital (Brietal) by injection into a tail vein.

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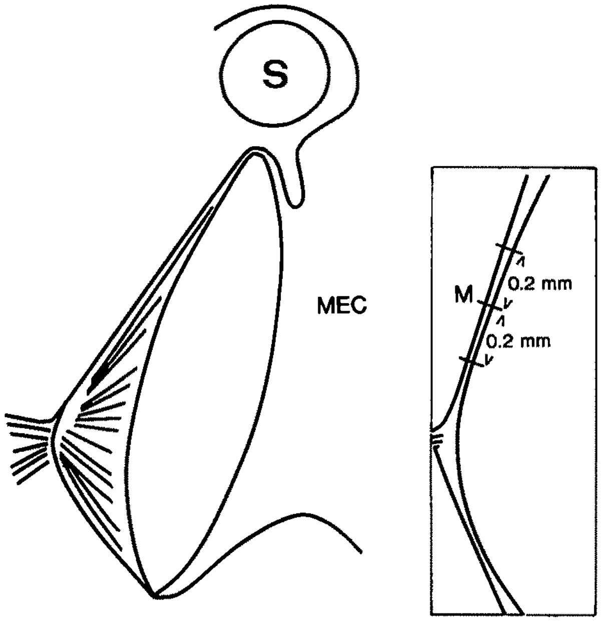


Fig 1. Schematic of round window membrane, in round window niche, showing points of measurement used in study. S — stapedial artery, MEC — middle ear cavity, M — midpoint.

SURGICAL PROCEDURES

Serous otitis media was provoked in 25 rats by plugging the eustachian tube of the left ear via a ventral midline incision.[7] A small hole was drilled in the floor of the tympanic bulla, and through this a gutta-percha plug (De Trey, Switzerland) was inserted hermetically into the tympanic orifice of the eustachian tube. Bilateral POM was induced in the other 25 rats by splitting the soft palate along the midline, from the posterior free border to the hard palate.[8] Five animals from each of the two groups were painlessly killed 1, 2, 3, 4, and 6 weeks after the various operative procedures.

OTOMICROSCOPY

Prior to killing, the tympanic membranes (TMs) were inspected through an otomicroscope, and the TM status was charted with particular attention to the presence of effusion material, the vessels, and the position of the TM. On opening of the tympanic bulla, the color and consistency of the middle ear fluid were determined visually.

LIGHT AND ELECTRON MICROSCOPIC ANALYSIS

The bulla was dissected free and opened, and the MEC was filled with 3% glutaraldehyde in 0.1 mol/L cacodylate buffer with 4% polyvinylpyrrolidone added. The temporal bones were stored in the same fixative for at least 1 week before the RWM was dissected free from the temporal bone. Two methods were used in order to obtain the RWM specimens — either the biopsy technique or the en bloc technique.

Biopsy Technique. Under the otomicroscope the lateral wall of the bulla was fractured and removed with a scalpel. The promontorial mucosa and, in certain cases, mucosa covering the RWM niche were removed with a Beaver knife in order to visualize the RWM. Promontorial bone and the bone adjacent to the RWM niche were then removed by drilling until less than 1 mm was left. The remaining bony rim with the RWM was then fractured and dissected free and further prepared for histology.

En Bloc Technique. In some cases, a larger piece containing the entire round window niche area, including the stapedial artery, was removed from the temporal bone with a scalpel. The piece of tissue was then transferred and left in the original fixative for at least 24 hours. The specimens were then decalcified in 10% ethylenediaminetetraacetic acid in 0.1 mol/L cacodylate buffer, pH 7.3, for 2 to 3 weeks. Some temporal bones were decalcified by means of a microwave oven (Miele M696) for 10 hours in the same solution.[13] With the latter technique the oven was operated at 2,450 MHz at an effect set initially at 450 W. The temperature was regulated to 37°C by a probe placed in the vial containing the tissue in the decalcifying medium. Once the required temperature was reached, the effect setting was changed to 150 W until the tissue had softened.

After the two techniques described above, the dissection procedure to obtain the RWM was performed in a petri dish. The specimens were irrigated in the original glutaraldehyde solution and then postfixed in 1% osmium tetroxide, dehydrated in increasing concentrations of acetone, and embedded in Polybed (812; epoxyresin; Poly Science Inc). The specimens were sectioned with an LKB ultramicrotome. Semithin sections (1 μm) for light microscopy were routinely stained with toluidine blue. For electron microscopy, ultrathin sections (80 nm) were contrasted with uranyl acetate and lead citrate. The sections were examined in a JEOL 100 CX transmission electron microscope.

ANALYSIS OF ROUND WINDOW MEMBRANE THICKNESS

The conically shaped RWM was sectioned vertically from the outer to the inner bony attachment. Two or three consecutive sections from each RWM were used from each time interval. The length of the sectioned membrane was divided into four portions (Fig 1). The first point was the midpoint; the second and third points were set at 0.2 mm from each side of the midpoint. By using a micrometer scale in the ocular, the total thickness of the RWM, as well as the thickness of its outer, middle, and inner layers, was calculated at the three measuring points. At each time

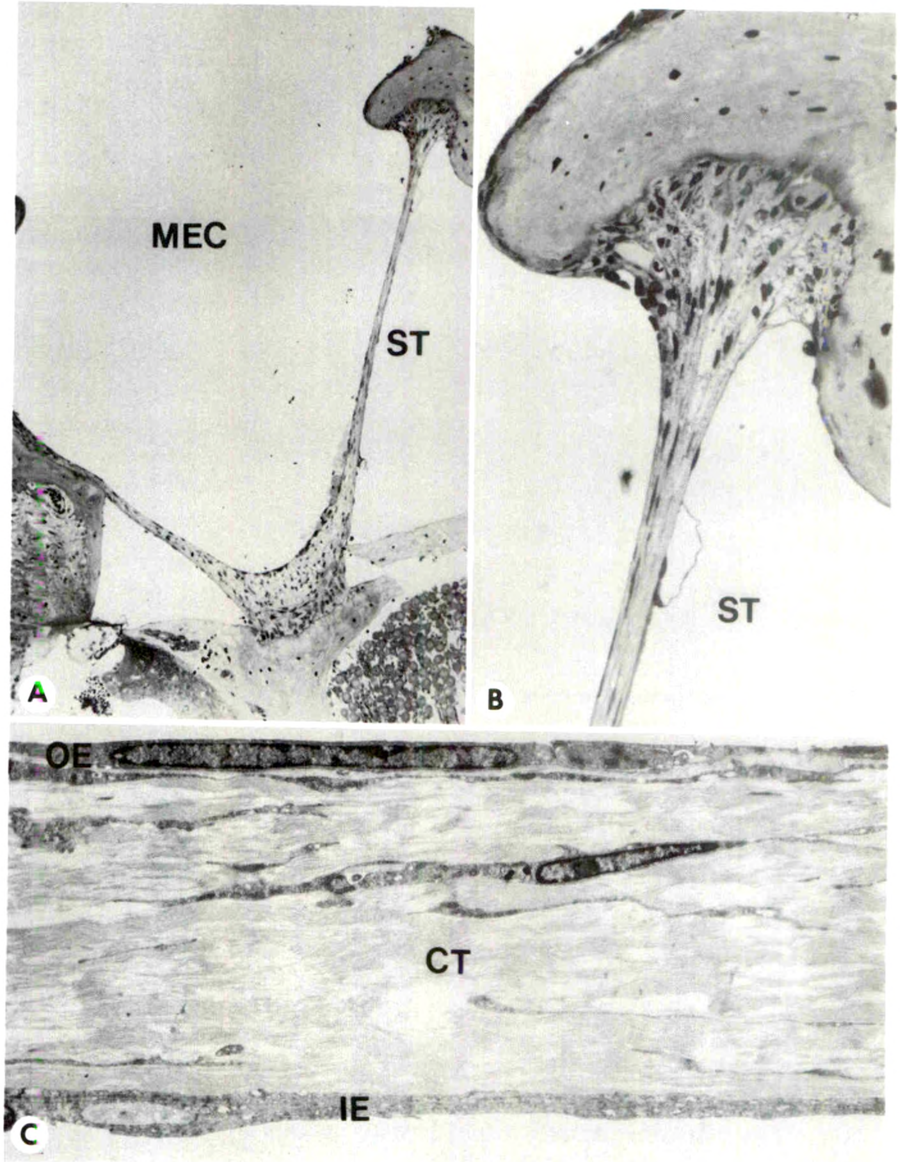


Fig 2. Normal round window membrane of rat. A) Light micrograph of vertical section (original x10). Note conical shape and short distance to inner ear. MEC — middle ear cavity, ST — scala tympani. B) Detail of triple-layer membrane, close to outer bony attachment area (original x40). ST — scala tympani. C) Electron micrograph (original x2,500). CT — connective tissue, OE — outer epithelium, IE — inner epithelium.

interval (1, 2, 3, 4, and 6 weeks), the median, first, and third quartiles were determined in the SOM and POM groups.

RESULTS

The conically shaped RWM of the rat is located in a niche situated at the superoposterior portion of the promontory, with the stapedial artery passing along the "roof" of the niche.[14] The bony framing of the round window niche does not allow direct inspection of the RWM, either from the external auditory canal or through the opened tympanic bulla. The RWM consists of three layers: a layer of flat epithelium lining the MEC, a middle layer of connective tissue, and an inner layer facing the perilymphatic space that consists of a single layer of mesothelial cells.

NORMAL RAT ROUND WINDOW MEMBRANE

The normal RWM is shown in Fig 2.

Outer Layer. The epithelium lining the MEC consisted of a single layer of squamous cells that were contiguous with the promontorial epithelial cells. The latter adhered to each other by fingerlike projections with tight junctions close to the epithelial surface (Fig 3). A basement membrane separated the outer layer from the middle connective tissue layer. The epithelium of the normal rat RWM contains no ciliated cells. The epithelial cell surface exhibited microvilli, most frequently close to the cell borders (Fig 3).

Middle Layer. The bulk of the rat RWM, the connective tissue layer, contained fibroblasts, fibrocytes, collagen bundles, blood vessels, and nerves (Fig 2C). The blood vessels (and the nerves) were located close to the bony attachment of the RWM (Fig 2B). Elastic fibers were sparse or absent. Collagen fibers occupied most of the middle layer. The

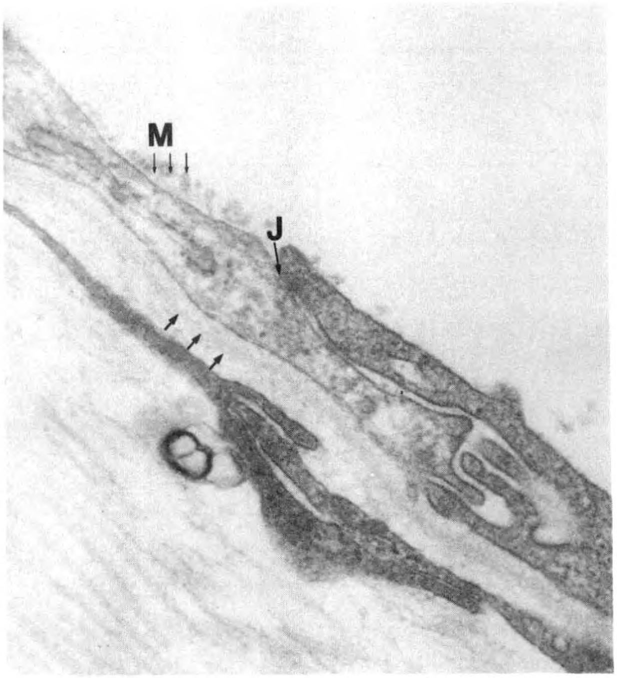


Fig 3. Electron micrograph of normal round window membrane of rat, showing detail of outer epithelium (original x30,000). Note fingerlike folds, microvilli (M), tight junction (J), and basement membrane (arrows).

distribution of the fibers was mostly vertically (longitudinally) arranged in the core of the membrane, although transversely sectioned fibers were also common. The latter occurred particularly close to the outer as well as the inner epithelium. The fibrocytes were the predominant cells in the connective tissue layer, forming long, thin cytoplasmic extensions. Fibrocytes were frequently present near the outer bony attachment area.

Inner Layer. Facing the perilymphatic space was a single layer of mesothelial cells with long, thin cytoplasmic extensions, sometimes overlapping each other. Cytoplasmic vesicles were found in these slender extensions close to their two surfaces. The inner epithelium was connected with desmosomes and less frequent tight junctions. No basement membrane was identified.

The thickness of the RWM was estimated to be 13 μm (12.5 to 15), of which the outer, middle, and inner layers occupied approximately 5%, 90%, and 5%, respectively (Fig 4).

SEROUS OTITIS MEDIA

Light micrographs of the RWM in SOM are shown in Fig 5.

One and Two Weeks. The epithelial cells lining the MEC displayed swollen and rounded nuclei, contrasting with those of the normal outer epithelium. Notably, there were more numerous microvilli on the epithelial cell surface facing the MEC. In particular, many microvilli appeared on the surface of epithelial cells overlying blood vessels. In the middle layer — and especially in close proximity to the epithelium — increased numbers of fibroblasts were seen. The appearance of the connective tissue layer varied, being either normal or showing a slight edema or a more dense collagen layer. The inner epithelium was unchanged or displayed a less electron-dense, sometimes slightly swollen cytoplasm.

Three Weeks. The outer epithelial cells were still flat. The connective tissue contained abundant fibroblasts, distributed mostly in the subepithelial space adjacent to the outer layer. Compared with the normal RWM, the collagen fibrils were more densely packed, and they were arranged longitudinally.

Six Weeks. The outer epithelium was similar to that at 1 and 3 weeks. The inner epithelium was slightly more swollen than that of the normal RWM. Similar to those at 1 week, the collagen bundles were more loosely arranged than at 3 weeks. In one specimen, an accumulation of elastic fibers was identified. This specimen also exhibited thicker collagen fibrils and abundant fibroblasts (Fig 6).

PURULENT OTITIS MEDIA

Findings in POM are shown in Fig 7.

One Week. The outer epithelial cells had become rounded, exhibiting microvilli all over the cell surface. Subepithelial edema was seen. The connective tissue layer displayed no major changes, other than a somewhat looser arrangement of the collagen fibrils. The cells of the inner epithelium exhibited, as in the SOM group, a less dense cytoplasm. In the subepithelial space an increased vascularization and an invasion of inflammatory cells, mainly polymorphonuclear neutrophils (PMNs), were noted.

Two Weeks. The structure appeared similar to that at 1 week, although a tendency of the collagen fibrils to aggregate was noted.

Three Weeks. The outer epithelium now was dominated by cuboid cells and cylindric cells. Some of the latter were ciliated. Goblet cells were frequently seen. Inflammatory cells, mainly PMNs but also lymphocytes, occurred in the subepithelial space. Some blood vessels were clogged with numerous red blood cells. No obvious changes in the connective tissue layer were seen.

Six Weeks. The epithelium facing the MEC showed a cuboid and cylindric epithelium containing both ciliated and goblet cells. The subepithelial space was loosely arranged, exhibiting several inflammatory

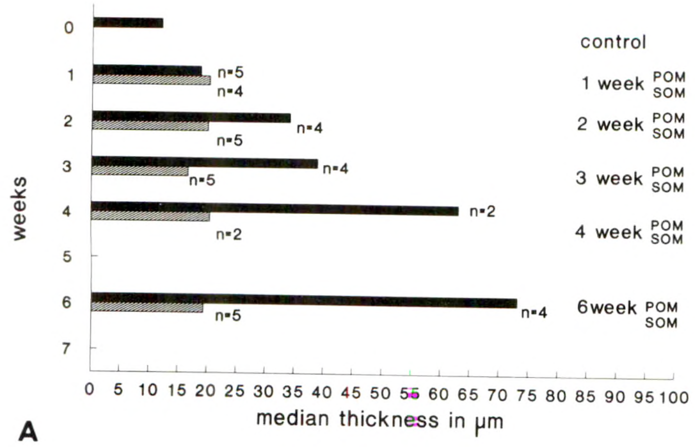
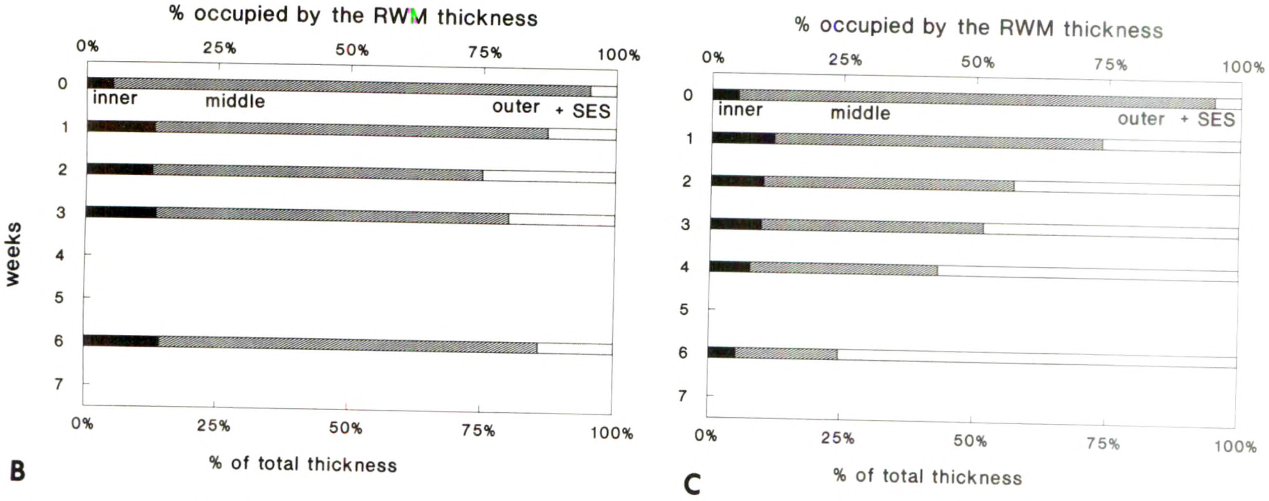


Fig 4. Round window membrane (RWM) measurements. A) Median thickness of RWM in normal, serous otitis media (SOM), and purulent otitis media (POM) conditions at various intervals after induction of inflammatory conditions (1, 2, 3, 4, and 6 weeks). Median thickness of RWM in POM increased from 20 µm at 1 week to about 70 µm at 6 weeks. In SOM group there was increase in thickness already at 1 week, which persisted and remained stable throughout study. B,C) Relative thickness, in percent, of various layers (inner, middle, and outer with subepithelial space [SES]) of RWM in controls (day 0) and in pathologic conditions at 1, 2, 3, 4, and 6 weeks. B) SOM. C) POM.



cells, including frequent PMNs, many lymphocytes, plasma cells, and a few macrophages. Dilated vessels were seen not only close to the bony attachment of the RWM, but also in its midportion. The connective tissue showed more fibroblasts than at earlier time points (Fig 8). In one membrane a polyplike structure was seen (Fig 9). In its stroma, multinucleated giant cells were found. The epithelium was dominated by cylindric cells with cilia.

ROUND WINDOW MEMBRANE THICKNESS IN SEROUS AND PURULENT FORMS OF OTITIS MEDIA

The thickness of the RWM in SOM and POM animals is shown in Fig 4. In the SOM group the RWM had thickened slightly after 1 week, but did not change further throughout the observation period. In POM, the median thickness increased with time from 20 µm at 1 week to roughly 70 µm at 6 weeks. Relatively, the outer layer with adjacent subepithelial space became more and more dominant. The connective tissue layer predominated in the SOM RWM, but exhibited an almost normal appearance. The changes at 2 to 3 weeks were correlated to the subepithelial space immediately beneath the outer epithelium and reflected proliferation of fibroblasts.

DISCUSSION

In our understanding of inner ear disturbances, whether transient or permanent, following various conditions and treatments in the MEC, the RWM as a portal to the inner ear has attracted increasing interest.[15,16] Quantitative and qualitative changes in the RWM structure have been assumed to influence the passage of various substances through the RWM. Several studies have been performed, charting the structure and ultrastructure of the RWM in animal models as well as in human preparations.[15,17-22] Likewise, tracer studies under normal and pathologic conditions have increased our knowledge of the RWM as a barrier between the middle and inner ear.[15,23-29] Sahni et al[19] reported that the RWM thickness increased during otitis media, to reach its thickest in chronic otitis media. Whether an increased RWM thickness impairs or facilitates permeation through the membrane is, however, still not known. Recently, Ikeda et al[29] showed that exposure of the chinchilla RWM to *Streptococcus pneumoniae* enhanced its permeability in the acute stage, whereas in the re-

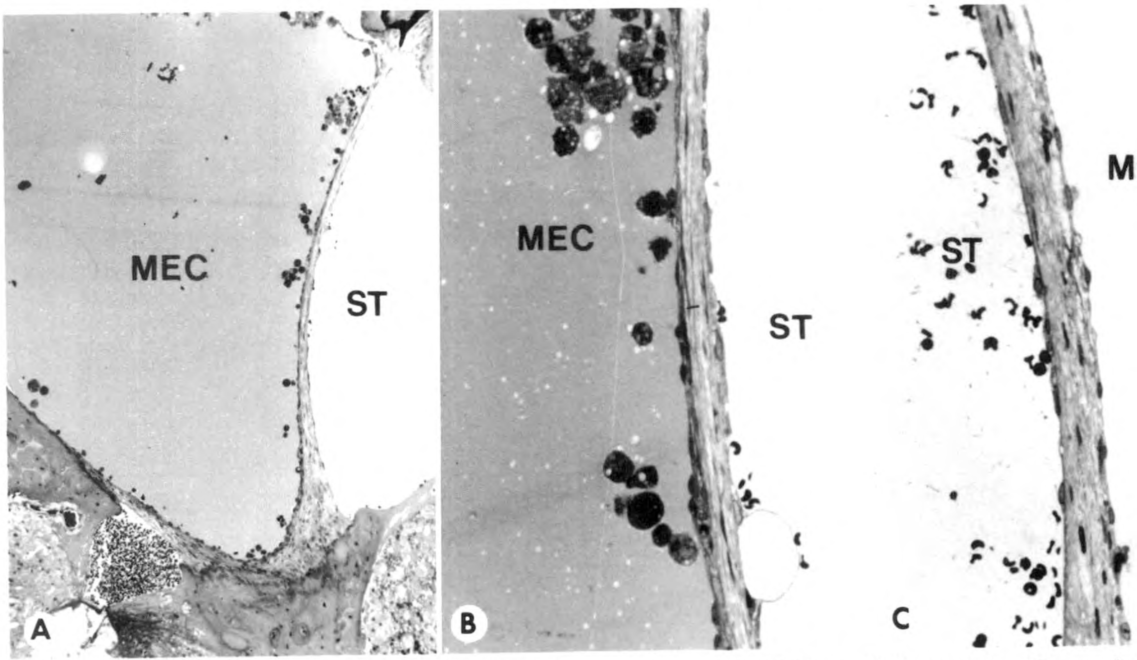


Fig 5. Light micrographs of round window membrane in serous otitis media. MEC — middle ear cavity, ST — scala tympani. A) Overview after 1 week (original x10). B) Detail at 1 week (original x40). C) At 6 weeks (original x40).

solved stage, permeability decreased. In both conditions the RWM was thickened, although differing with regard to the content of inflammatory cells and the appearance of the outer epithelial lining.

In the present study the rat model was used as a means to determine the structural changes taking place in the RWM in two well-defined types of otitis media: SOM, representing a noninfectious condition, and POM, an infectious inflammatory condition.[12] The basic triple-layered structure of the conically shaped rat RWM resembles that of other species, but its overall thickness is only one sixth of that in the human. Another difference concerns its content of elastic fibers, which are present in the human RWM,[18,21] in the rhesus monkey,[25] and in the cat,[20] but almost totally lacking in the rat RWM.

The RWM in SOM and POM was found to differ radically not only in thickness, but also structurally. Under the light microscope the RWM in the SOM group did not show any major changes during the observation period. The POM group, however, displayed pronounced structural alterations of the RWM, confined mainly to the epithelium lining the MEC and the occurrence of inflammatory cells in the subepithelial space adjacent to the outer epithelium. The flat outer epithelium gradually changed to a cuboidal and cylindric epithelium, with ciliated and goblet cells. In general, goblet cells occurred prior to ciliated cells. The findings agree with an earlier study on the rat pars flaccida in which POM caused metaplasia of the epithelial lining facing the middle ear cavity while in SOM the epithelium did not change.[10]

In the SOM group the RWM exhibited an almost normal structural appearance, although its thickness increased moderately during the first week and continued to do so throughout the study. At an ultrastruc-

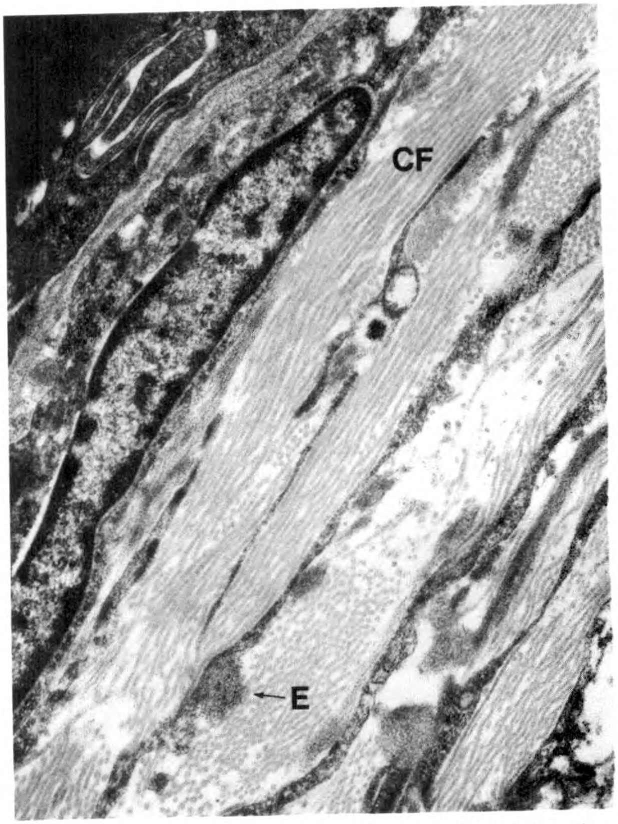


Fig 6. Electron micrograph showing elastic fibers (E) and collagen fibers (CF) in round window membrane in serous otitis media at 6 weeks (original x10,000).

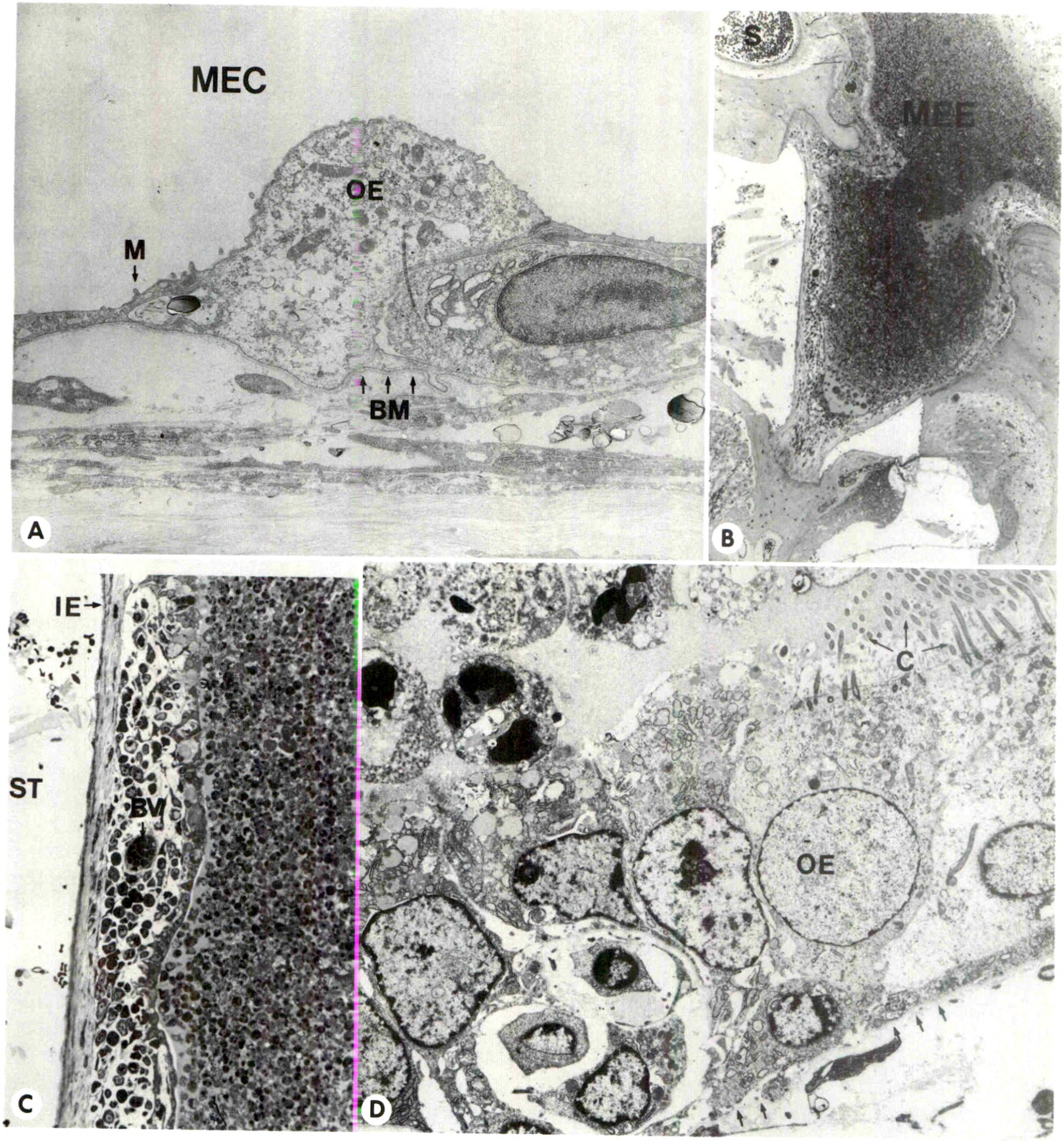


Fig 7. Round window membrane in purulent otitis media. A) Electron micrograph at 1 week (original x5,000). OE — outer epithelium, M — microvilli, BM — basement membrane, MEC — middle ear cavity. B) Light micrograph at 6 weeks (original x10). S — stapedial artery, MEE — middle ear effusion. C) Detail of B at higher magnification (original x40). ST — scala tympani, IE — inner epithelium, BV — blood vessel. D) Electron micrograph at 6 weeks (original x2,500). OE — outer epithelial cell, C — cilia, arrows — basement membrane.

tural level, some outer epithelia seemed rounded and the epithelial cells exhibited microvilli all over the surface, not only confined to the cell borders. The most striking changes in the SOM vis-à-vis normal and POM RWMs were seen in the connective tissue layer at an ultrastructural level. Although the relative thickness of the three basic layers was similar to that in the normal RWM, an increased number of fibroblasts was seen throughout the middle layer. The collagen fibrils were densely aggregated, and in one case elastic fibers were seen in the middle core and close to the inner epithelium. The collagen fibrils seemed thicker than normal. The increased fibroblast activity and collagen fibril pattern indicate a reorganization of the connective tissue. An increased content of collagen fibers may affect the physical prop-

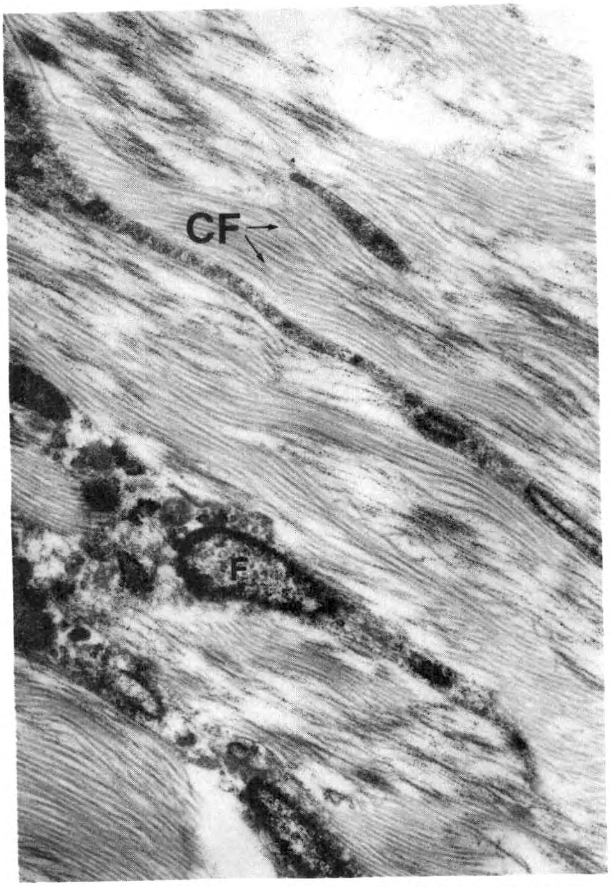


Fig 8. Electron micrograph of round window membrane representing connective tissue layer in purulent otitis media at 6 weeks (original x10,000). F — fibroblast, CF — collagen fibers.

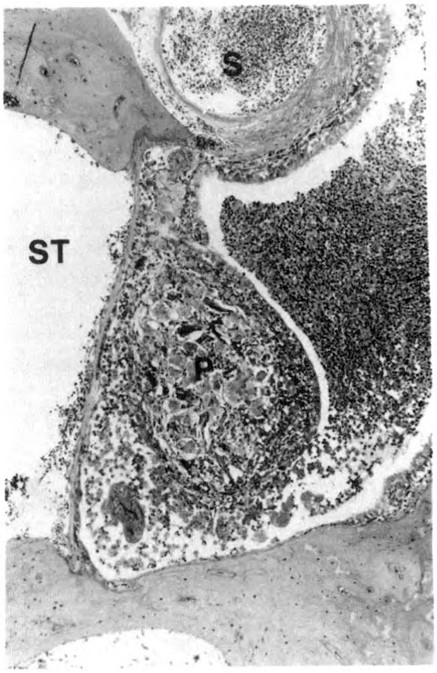


Fig 9. Light micrograph of round window membrane in purulent otitis media at 6 weeks, showing mucosal polyp (P) in round window niche (original x10). ST — scala tympani, S — stapedial artery.

erties of the RWM, including its permeability.

In the POM model used in the present study the microbiologic flora was dominated by gram-negative bacteria, mainly *Escherichia coli*.[12] These bacteria produce endotoxins that can facilitate permeability through the membrane. Endotoxins applied on the rat RWM have been shown to pass through the membrane and affect the inner ear function.[30] The changed epithelium in POM could act as a defensive barrier. Interestingly, the infection not only increased the epithelial height but also caused mucosal folds, and organized effusion material and polyp formation in the round window niche, which may prevent access to the round window area. The subepithelial space was gradually invaded by various inflammatory cells forming an additional line of defense. To meet with the increased functional demands, there was an ingrowth of vessels in the connective tissue layer.

The interpretation of the swollen inner epithelial cells seen in both groups is still obscure. In a recent study we showed that the MEE in SOM is very protein-rich[31] and thus hyperosmolar. Similarly, Juhn et al[28] showed that serous MEEs in humans are hyperosmolar and cause a shift of water from the perilymphatic space. It is conceivable that the changed inner epithelium may reflect an increased absorptive activity from the perilymph.

In the present study we have characterized the structure of the rat RWM in two distinct types of otitis media, SOM and POM. The two inflammatory conditions caused different structural changes to the RWM, which may in different ways influence passage between the middle ear and inner ear. The observations have prompted electrophysiologic studies, now in progress, to evaluate the inner ear function in these otitis media models (paper in preparation). Furthermore, the study showed that high microscopic resolution is needed to reveal the structural alterations present in these inflammatory conditions, especially as regards the changes associated with the connective tissue layer and epithelial membrane surface.

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IMAGING CASE STUDY OF THE MONTH

COMPUTED TOMOGRAPHIC DIAGNOSIS OF RADIATION-INDUCED INTERNAL CAROTID ARTERY THROMBOSIS

YANCU HERTZANU, MD FERIT TOVI, MD

BEER-SHEVA, ISRAEL

INTRODUCTION

The local effects of radiotherapy in the head and neck are well known. These include radiation dermatitis, mucositis, xerostomia, dental caries, and osteonecrosis. However, radiation-induced damage to the carotid artery is hardly discussed in the head and neck surgery literature. From this vantage point we wish to describe the computed tomography (CT) findings of internal carotid artery thrombosis in a patient with a history of irradiation to the neck.

CASE REPORT

A 52-year-old man was referred to our medical center because of a mass in the left side of the neck and severe facial edema. Three years previously he had undergone elsewhere a supraglottic laryngectomy and right radical neck dissection for a supraglottic squamous cell carcinoma. Postoperative radiotherapy had been indicated on detection of residual tumor at the base of the tongue. The patient then received external irradiation of 56 Gy.

On admission, the general physical and neurologic examinations yielded normal findings. Marked protrusion of the left pharyngeal wall and multiple fixed and enlarged cervical lymph nodes were detected on otorhinolaryngologic examination. The routine blood count and chemistry were normal. Radiography of the chest showed no abnormality.

On postcontrast CT, both common carotid arteries appeared normal, and the internal jugular veins could not be visualized in the lower neck. In the middle part of the neck a large, nonhomogeneous mass involving the left parapharyngeal space was found. The right external and internal carotid arteries were well demonstrated. The left internal carotid artery was visualized adjacent to the pharyngeal mass. The left jugular vein could not be demonstrated (Fig 1). At the level of the upper neck both parapharyngeal spaces were well preserved. Two round rims, enhancing low-density structures, were seen in the anatomic location of the major left cervical vessels. These features were compatible with thrombosis of both the left internal carotid artery and the left internal jugular vein. The right internal carotid artery was normal. The right internal jugular vein appeared to be thrombosed (Fig 2).

Accordingly, a diagnosis of thrombosis of the left internal carotid artery and internal jugular vein was considered. A cytologic diagnosis of squamous cell carcinoma was obtained from a fine-needle biopsy of the left cervical lymph nodes, and chemotherapy was initiated.

DISCUSSION

Apart from severe atherosclerosis, two factors may be considered in the etiopathogenesis of internal carotid artery occlusion in patients suffering from head and neck carcinoma.[1] The first is the involve-

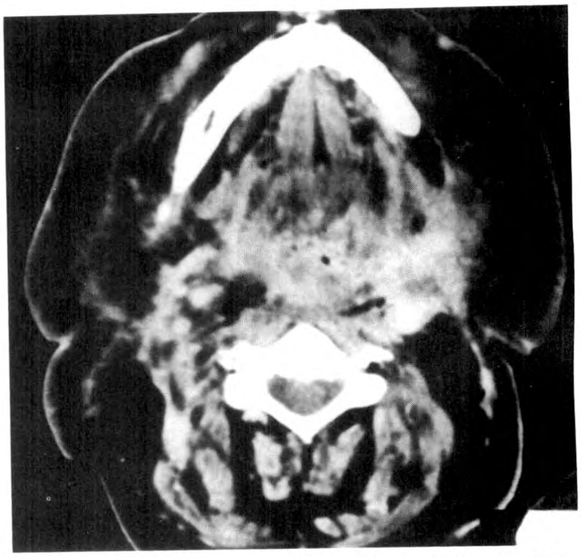

Fig 1. Computed tomogram of middle level of neck after intravenous administration of contrast medium. Nonhomogeneous mass is noted in front of left internal carotid artery.

From the Departments of Radiology (Hertzanu) and Otolaryngology (Tovi), Soroka Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

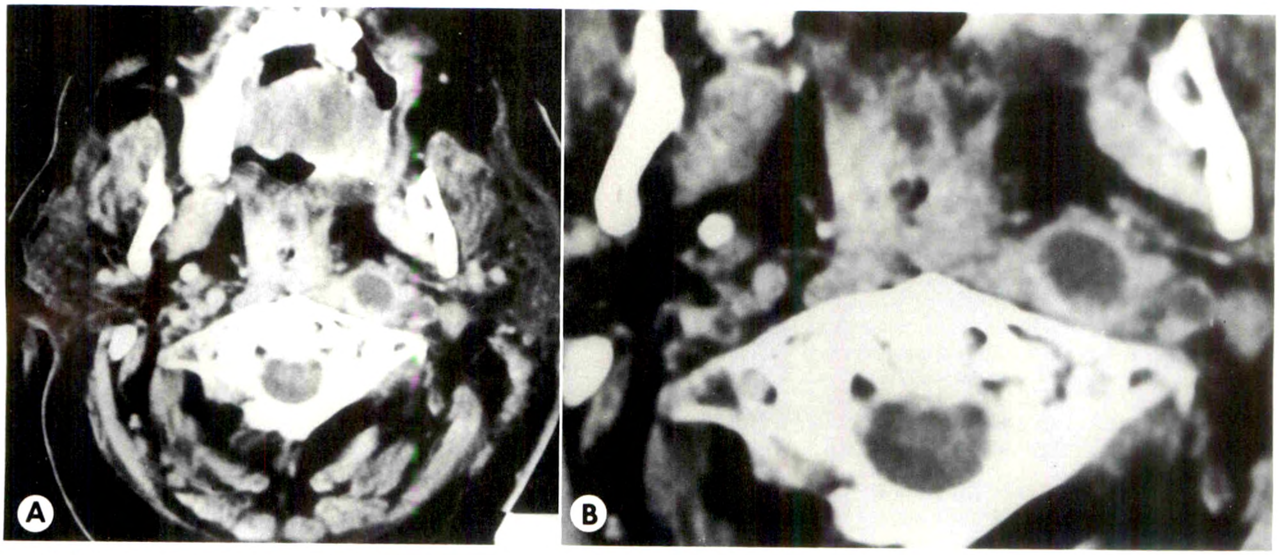

Fig 2. Computed tomograms of upper level of neck. A) Thromboses of left internal carotid artery and left internal jugular vein are seen. B) Magnification of A.

ment of the artery by a tumoral process, and the second consists of radiation-induced thrombosis. In the present case the absence of atherosclerotic changes in the bifurcation of both carotid arteries as demonstrated by CT almost excludes this as a cause of the left internal carotid artery occlusion. The possibility of tumoral occlusion of this artery can also be excluded according to its normal CT appearance at the level of the tumoral process. Therefore, the previous irradiation should be considered in the pathogenesis of this condition.

Radiation-induced damage to the arteries has been studied experimentally and clinically. In this condition histopathologic changes of the arterial wall are similar to those occurring in atherosclerotic disease.[2-4] These changes have been shown in the coronary arteries of patients irradiated for chest lymphomas, in renal arteries irradiated for retroperitoneal malignancies, in iliac arteries irradiated for gynecologic malignancies, and in subclavian and axillary arteries.[5,6]

Of the patients irradiated for head and neck carcinomas, 25% to 30% had internal carotid artery damage.[7] In one series the incidence of arterial wall changes in irradiated patients was found to be fivefold higher than in the control group.[8] Radiation-induced arterial wall changes occurred at doses of 40 to 50 Gy.[9] Carotid artery changes occurred after an interval of 28 months to 5 years.[8,9] The spectrum of the pathology of irradiated carotid arteries includes disruption of the arterial wall, stenosis, and complete occlusion. Blowout of the artery is the most disastrous complication.[6]

The central nervous system manifestations of radiation-induced carotid artery damage vary from transient ischemic attacks to devastating cerebral infarction. However, in the absence of embolic processes or in the presence of compensatory Willis circulation, neurologic damage may not occur. Diverse methods of diagnosis are available for the recognition of carotid artery occlusion. The availability of noninvasive methods limits the use of angiography only to patients who are candidates for endarterectomy.

Computed tomography is a recommended technique in the follow-up of patients with head and neck malignancy, as well as for the imaging of the major vascular channels in the same area.[10,11]

The ability of a CT scan to identify a very low concentration of injected contrast agent is used to determine various carotid artery abnormalities, including degenerative atheromatous changes, aneurysm formation, subintimal hemorrhage, and disruption.

In the present case, thrombosis of both jugular veins — that of the right being related to neck dissection — was clearly indicated by the typical CT aspect of these vessels.[12] This consists of a low-density lumen related to the thrombus, surrounded by a rim enhancement that corresponds to the filling of the vasa vasorum by contrast medium.

In conclusion, radiation-induced internal carotid artery thrombosis should be considered in the follow-up of irradiated head and neck cancer patients. The survival of these patients today is prolonged as a result of advancing techniques of therapy. Therefore, we expect an increase in the incidence of radiation-induced carotid artery damage. Follow-up CT appears to be very helpful in the detection of this vascular complication.

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6TH INTERNATIONAL CONGRESS ON INTERVENTIONAL ULTRASOUND

The 6th International Congress on Interventional Ultrasound will be held Sept 7-10, 1993, in Copenhagen, Denmark. For further information, contact Christian Noisse, Congress Secretary, Department of Ultrasound, Herley Hospital, University of Copenhagen, DK-2730 Herley, Denmark.

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SAN FRANCISCO OTOLOGY UPDATE — 1993

A comprehensive review of contemporary otology and neurotology is offered for the practicing otolaryngologist or resident in training. It will be held at the Ritz-Carlton Hotel, San Francisco, California, on November 4-6, 1993. For further information, contact UCSF Extended Programs in Medical Education, Room LS-105, San Francisco, CA 94143-0742; phone 415-476-4251.

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PATHOLOGY CONSULTATION

HYALINIZING TRABECULAR ADENOMA OF THE THYROID GLAND

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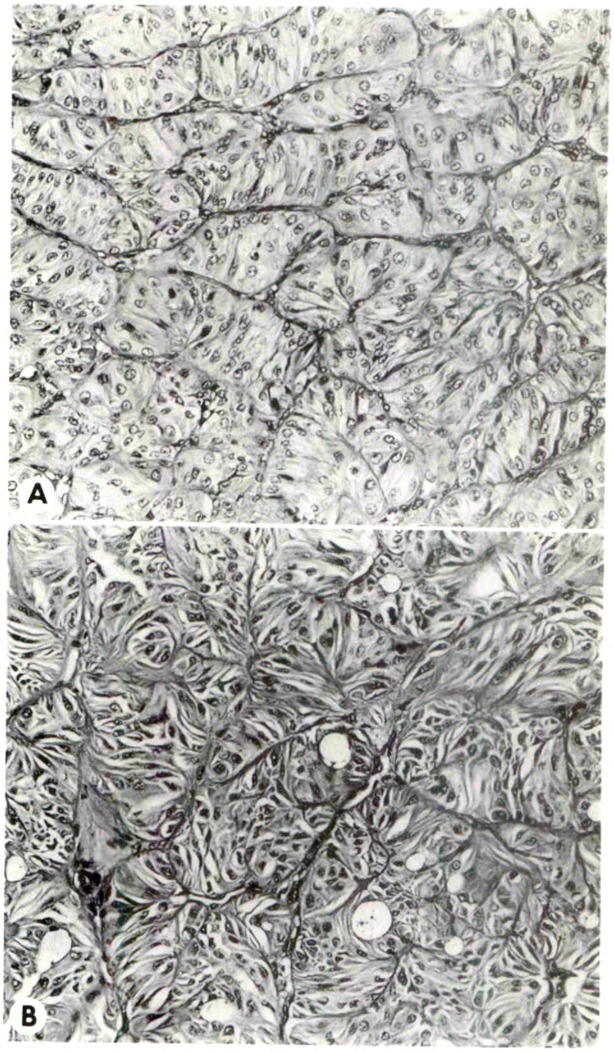
Hyalinizing trabecular adenoma is a follicular-derived tumor of the thyroid gland with a strong predilection for women. In the reported cases, the biologic course has been benign and without recurrences. The most important histologic differential diagnosis is medullary carcinoma, particularly the encapsulated form of that neoplasm.

The hyalinizing trabecular adenoma, also known as paragangliomalike adenoma, is a recent addition to the taxonomic classifications of neoplasms of the thyroid gland.[1,2] First described in 1987 by Carney et al,[1] the tumor is an uncommon lesion of the thyroid gland, but its importance resides in its possible confusion with other neoplasms, particularly medullary carcinoma of the thyroid and paraganglioma.

The hyalinizing trabecular adenoma is noteworthy for its female preponderance and benign clinical course. In the combined cases of Carney et al[1] and Bronner et al,[2] there were 22 women and 1 man. The average age at thyroidectomy was 46 (range 27 to 72). In gross appearance, these two reports describe a single dominant tumor (0.3 to 4 cm) or a tumor in association with other thyroid nodules. It is solid, yellow-tan, and encapsulated or circumscribed. In microscopic appearance there is a solid, lobulated growth pattern with a trabecular or alveolar arrangement and alignment of polygonal, oval, and spindle cells with perinuclear clearing and nuclear pseudoinclusions. There is hyalinization of intertrabecular stroma and "mummification" of adjacent epithelial cells. There are pseudofollicles and true follicles. The tumors were found to be calcitonin- and neurofilament-negative, thyroglobulin-positive, and occasionally focal S-100 protein antigen–positive. They were keratin-positive and negative for amyloid. They may show neuroendocrine differentiation. The biologic behavior is benign. The 23 cases show no recurrences or metastases to date.

Despite the rather distinctive histopathologic appearance of the hyalinizing trabecular adenoma (see Figure), it is mandatory to exclude medullary carcinoma of the thyroid, especially the encapsulated type



Hyalinizing trabecular adenoma of thyroid gland from 34-year-old woman (H & E). A) Trabecular growth pattern of tumor with organoid clustering of cells. B) Spindle cell forms of adenoma's cells. Note follicles and pseudofollicles. (Continued on next page.)

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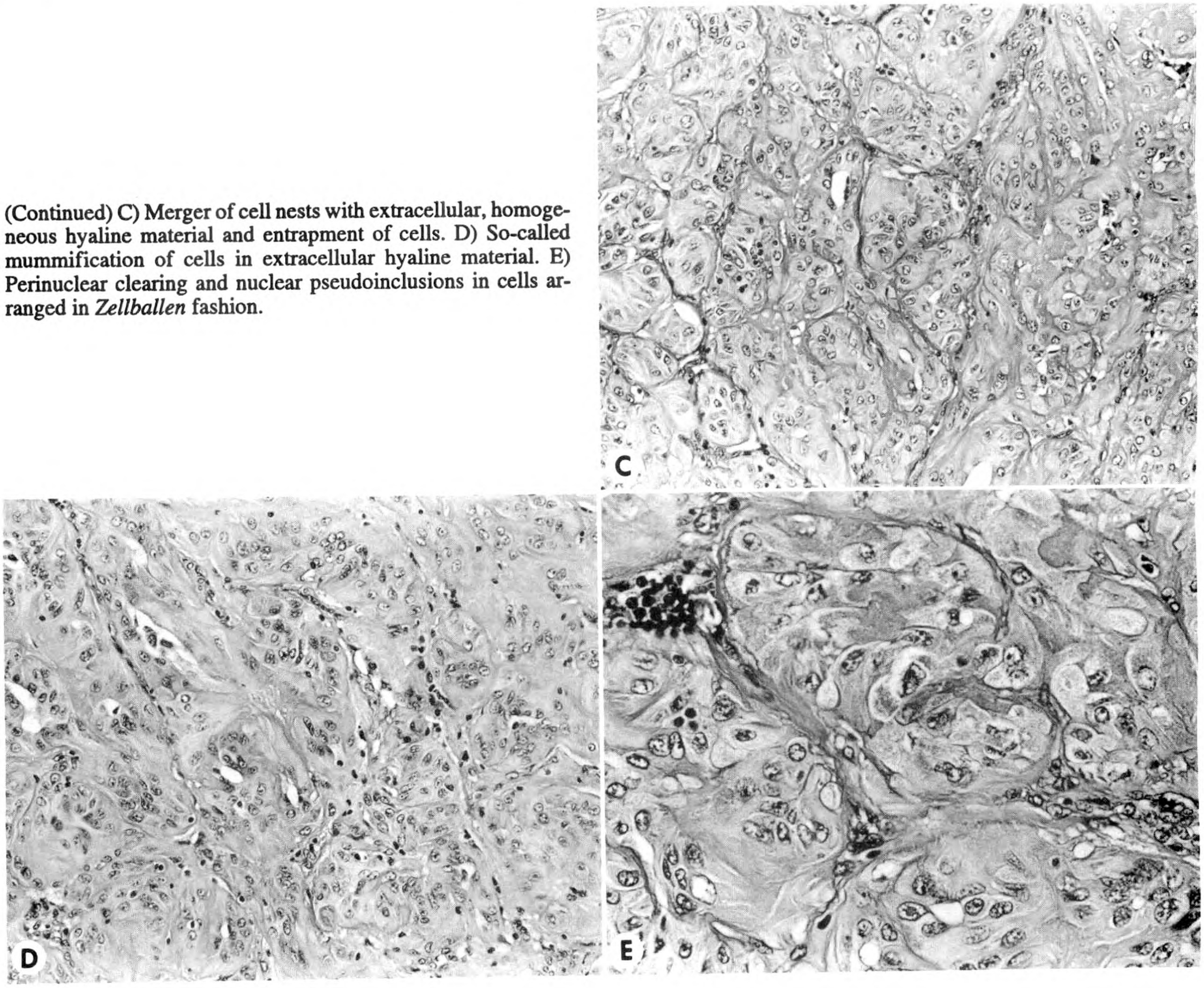
REPRINTS — John G. Batsakis, MD, Dept of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

(Continued) C) Merger of cell nests with extracellular, homogeneous hyaline material and entrapment of cells. D) So-called mummification of cells in extracellular hyaline material. E) Perinuclear clearing and nuclear pseudoinclusions in cells arranged in *Zellballen* fashion.



of that neoplasm.[2] A nonamyloid stroma, immunoreactivity with antibodies against thyroglobulin, and a nonreactivity for calcitonin should clarify the issue.

Katoh et al[3] have reported what appears to be a variant form of the hyalinizing trabecular adenoma. They examined three tumors that showed, in addition to immunocytochemical staining for thyroglobulin and keratin, an immunoreactivity for chromogranin and neuron-specific enolase. Further neuroendocrine differentiation was exhibited by one of the three tumors by an isolated cell immunoreactivity for somatostatin, a cytoplasmic argyrophilia, and electron-dense endocrine granules in the cytoplasm.

Since intrathyroid paragangliomas have not been conclusively proven to exist, they are less of a problem.[2,4] Thyroglobulin reactivity and the finding of follicles and colloid in the hyalinized trabecular adenoma serve as differential diagnostic points.

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Long-Term Results and Indications in Otology and Otoneurosurgery

R. Charachon and E. Garcia-Ibanez, editors. Hard cover, illustrated, indexed, 596 pages, 1991. Kugler and Ghedini, Amsterdam, the Netherlands.

Most of the articles presented in this book were on the program of the Politzer Society conferences in Ibiza (1989) and Courchevel (1990). The topics represent the clinical experiences of eminent and internationally recognized otologists and otoneurologists, and provide wide-range coverage of exciting new developments in otology. Fifteen of the most commonly discussed otologic conditions are covered in a series of concise reports. Eighteen such reports cover the sole topic of cholesteatoma. Other topics include tympanic membrane and retraction pockets; materials used in the reconstruction of mastoid cavities; ossiculoplasty (not all problems are solved); use of the perforator, microdrill, and laser in otosclerosis surgery; facial reanimation techniques for facial palsy (too briefly covered); streptomycin perfusion in Meniere's disease; scintigraphy followed by preoperative embolization for glomus tumors; antigens of the inner ear other than collagen II; and other areas of otology. Rarely seen conditions, such as carcinoma of the ear and the effects of the acquired immunodeficiency virus on the hearing organ, are not covered.

The most stimulating in this text are the discussions found in the smoothly written and highly informative articles on Meniere's disease. The familiarity of the syndrome, its associated vertigo, the incidence of bilaterality, and other insights into the disease are presented in the 11 reports. An interesting correlation is made between the natural course and psychologic aspects of Meniere's disease and the endolymphatic shunt operation. This is particularly intriguing when the mechanism of the shunt procedure is considered: decompression, passive diffusion, and osmotic pull. As is pointed out in one of the articles, there still remains a great need for controlled study data to evaluate the long-term results of treatments for Meniere's disease and the endolymphatic shunt. Ironically, despite its title, the lack of long-term results is the consistent and primary deficiency in this book as a whole.

Most of the authors are European, although some are Asian and a few are American. The text should appease or, better yet, whet the appetite of the inquisitive reader. For the American reader, it provides the experience and results of others from a broad horizon. Other than the fact that some of the papers, such as those on cochlear implants, convey experiences rather than information, little is left wanting in this book. It will be informative reading for the practicing physician interested in reviewing clinical studies that are shaping the perspectives of modern otology. The editors are to be commended for the infrequency of errors in grammar and composition and for tendering a well-organized volume that may precipitate new research.

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Color Atlas of Anatomy

Johannes W. Rohen and Chihiro Yokochi. Second edition. Hard cover, indexed, illustrated, 469 pages, 1988. Igaku-Shoin, New York, NY.

Human anatomy has not overtly changed since the publication of the first significant medical illustrations, found in Andreas Vesalius' celebrated 1543 anatomic text, *De humani corporis fabrica libri septem*, commonly known as the *Fabrica*. (The artist was Titian's student Jan Stephan van Calcar.) Recognition that a knowledge of human anatomy is essential to any field of medicine has also remained constant since ancient times. What *has* changed is dramatically demonstrated in this atlas of anatomy, and that is how knowledge of human anatomy is graphically disseminated in medical literature. Other atlases have used schematic and semischematic drawings as well as black-and-white and color photographs to depict anatomic structures, but few have reached the height of this one in delineating the anatomy of the human body. From the head through the neck, trunk, upper extremities, organs of the thorax, abdomen and retroperitoneum, and on through the urogenital system and lower extremities, the structures and organs are clearly delineated in exquisite color photographs. Black-and-white photographs, as well as well-done schematic drawings, are prudently used to augment the color photographs.

Among the authors' purposes in presenting a second edition of their original 1983 atlas was to include photographs from newly dissected specimens along with important computed tomography scan figures and magnetic resonance images. The format of the two editions is essentially the same, that is, to present macroscopic anatomy from the regional and stratigraphic viewpoints whenever possible and present it didactically. The glory of this edition stems from the authors' appreciation of the consequences of the increase in the number of students in anatomy with a coinciding decrease in the number of cadavers available for study. To the extent such an imbalance can be compensated for, the authors have excelled. While obviously not of equal value to dissecting a cadaver, their macroscopic photographs transcend the more commonly seen painted photographs.

Any conscientious physician will appreciate the opportunity this volume gives to review general human anatomy; each specialist will, of course, be primarily interested in his or her field of expertise. The otorhinolaryngologist–head and neck surgeon should not be disappointed. Almost one third of this atlas deals with the anatomy of the head and neck, including the skull, cranial nerves, nose, eyes, dental areas, face, sinuses, ears, and other areas. It is a real pleasure to see rarely depicted ear structures.

This atlas should be on the library shelf of every public and higher-educational institution, as well as specifically that of medical and dental schools and in the personal libraries of all physicians. Although published in 1988, with some repetition from the 1985 edition, the material presented is still timely, relevant, pertinent, and superbly presented.

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Head and Neck Oncology Diagnosis, Treatment, and Rehabilitation

Ernest M. Myers, editor. Hard cover, illustrated, indexed, 558 pages, 1991. Little, Brown, Boston, Mass, $85.

This book clearly follows an outline format. While this approach facilitates easy reading and quick referencing, it also induces brevity in coverage of topics. It is stated in the introduction that algorithms have been strongly relied on in presenting the material in the text; so, too, has the visual impact of generous usage of tables, drawings, and photographs.

Beginning with an overview of the demographics, etiology, pathogenesis, diagnosis, treatment, and other aspects of head and neck cancer, the editor and his colleagues proceed to give capsulized current information about this condition, including the newest technology applied in diagnosis and treatment. For the sake of completeness, rehabilitation for hearing loss and vestibular dysfunction secondary to treatment for head and neck cancer should certainly be mentioned. The appropriateness of the depth of attention (37 pages) being devoted to audiologic assessments, including electronystagmography, in a text primarily addressed to the disease itself is, however, debatable.

Although accurate and clearly presented, it is doubtful that the information in this book is sufficient to satisfy the needs of physicians who are faced with the dilemma of managing patients with head and neck cancers. On the other hand, primary care physicians, nurses, therapists, caretakers, family members, and others who are in direct contact with these patients will be well advised to consult this book for its sensitive awareness of the unique emotional turmoil experienced by victims of head and neck cancer. The language is, for the most part, unencumbered by technical and medical terminology and can be easily understood by most of those conversant with this condition.

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Inner Ear Pathobiology

M. Ciges and A. Campus, editors. Volume 45 of *Advances in Oto-Rhino-Laryngology*, C. R. Pfaltz, series editor. Hard cover, illustrated, indexed, 224 pages, 1990. S Karger, Basel, Switzerland.

This small volume contains material presented at the Inner Ear Pathology Symposium in Granada, Spain, in September 1989. The 23 articles the text comprises are, for the most part, brief presentations of the most advanced techniques for morphologic, biologic, and pathologic studies of the inner ear. An exception is the first chapter of 46 pages, in which state-of-the-art methods for bringing biologic tissue from its in vivo state to in situ form in the microscope are discussed. Although not particularly new, this is a timely topic, since techniques for chemical preservation of specific inner ear structures differ from those used in other current studies in otopathology.

Another paper contradicts the 1933 work of Wilhelm Steinhausen, who demonstrated in the pike fish that the cupula was a watertight barrier swayed by endolymph. Later studies supported the thought that the cupula was attached to the roof of the ampulla and therefore was moved only by pressure. In the study presented in this book, scanning electron microscopy revealed that in the Mongolian gerbil the attachments to the roof of the ampulla (tegmen ampulla) are linked to the cupula by filaments. Thus, a surge of endolymph would displace the cupula except at its central attachment to the tegmen, which acts as a spring to return the cupula to its original position on cessation of the surge. Mechanically, then, the cupula attachments act as a fulcrum.

Other articles in this text address the biologic and pathologic aspects of the inner ear from a functional perspective. Especially relevant are those that discuss problems arising from ototoxicity and the aging process. Even though the toxic effects of cisplatin are well documented and are known to involve the organ of Corti, the microanalysis is especially interesting. This study revealed an insignificant increase in calcium levels in the base of the hair tufts and at the apical surface of the hair cell after the administration of cisplatin. Of more significance was that higher doses of cisplatin induced decreased calcium levels.

A number of other interesting and significant ototoxic manifestations are discussed in this book, such as the lack of potential damage with local application of ciprofloxacin, high-frequency hearing loss in patients with tinnitus, and others.

The clinical applications in this small volume are noteworthy, but not earth-shattering. It is, however, of great value to have basic research findings in otology, histology, and pathology that have clinical applications, and this volume offers several. It should be of value to both clinicians and basic researchers.

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